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THE METAZOAN PARASITES OF THE HETEROSOMATA OF THE GULF OF ST. LAWRENCE

III. COPEPODA PARASITICA[1,2]

KEITH RONALD[3]

Abstract

Four species and one subspecies of parasitic copepods are recorded and briefly described from the Heterosomata of the Gulf of the St. Lawrence, and the distribution of the hosts indicated.

The copepod parasites of marine fish are fairly well known from eastern waters through the work of Bere (1), who studied these in the Passamaquoddy region. Wilson (7, 8) recorded a copepod from Cape Breton Island in 1932, and several others in 1944. Stock (6) gave the first record of a parasitic copepod, *Chondracanthus cornutus* (= *Acanthochondria cornuta*) on *Pseudopleuronectes americanus* in eastern Canadian waters. Heller (2) recorded several parasitic Copepoda from the area of the present survey, but none were from the Heterosomata.

The three species of flatfish found carrying parasitic Copepoda on, or in, their bodies were the halibut, *Hippoglossus hippoglossus* (Linné, 1758), plaice, *Hippoglossoides platessoides* (Fabricius, 1780), and the winter flounder, *Pseudopleuronectes americanus* (Walbaum, 1792) (Fig. 1). These three species were part of a general survey made upon 660 specimens of seven species of flatfish. In the case of the Copepoda many were undoubtedly lost in handling on the boat and in the water so that no record is made in this paper of the over-all incidence.

The fish were examined externally with an illuminated magnifier. They were then washed with salt water and the water used passed through a series of sieves to remove any small ectoparasites. The branchial and oral cavities were examined for parasites. The gill filaments were removed and an examination made using the stereodissecting microscope. The copepods were separated from the other parasitic groups present and stored in 4% formalin. They were later examined superficially in this dilute preservative. For more detailed study they were heated in 10% potassium hydroxide; this left the sclerotized exoskeleton intact, but removed the softer parts of the body. The exoskeleton was dehydrated in 90% ethanol and mounted in "euparal, vert" on a microscope slide, with a cover slip firmly in contact with supporting glass chips.

[1] Manuscript received September 9, 1957.
[2] No. 63, Contributions du Département des Pêcheries.
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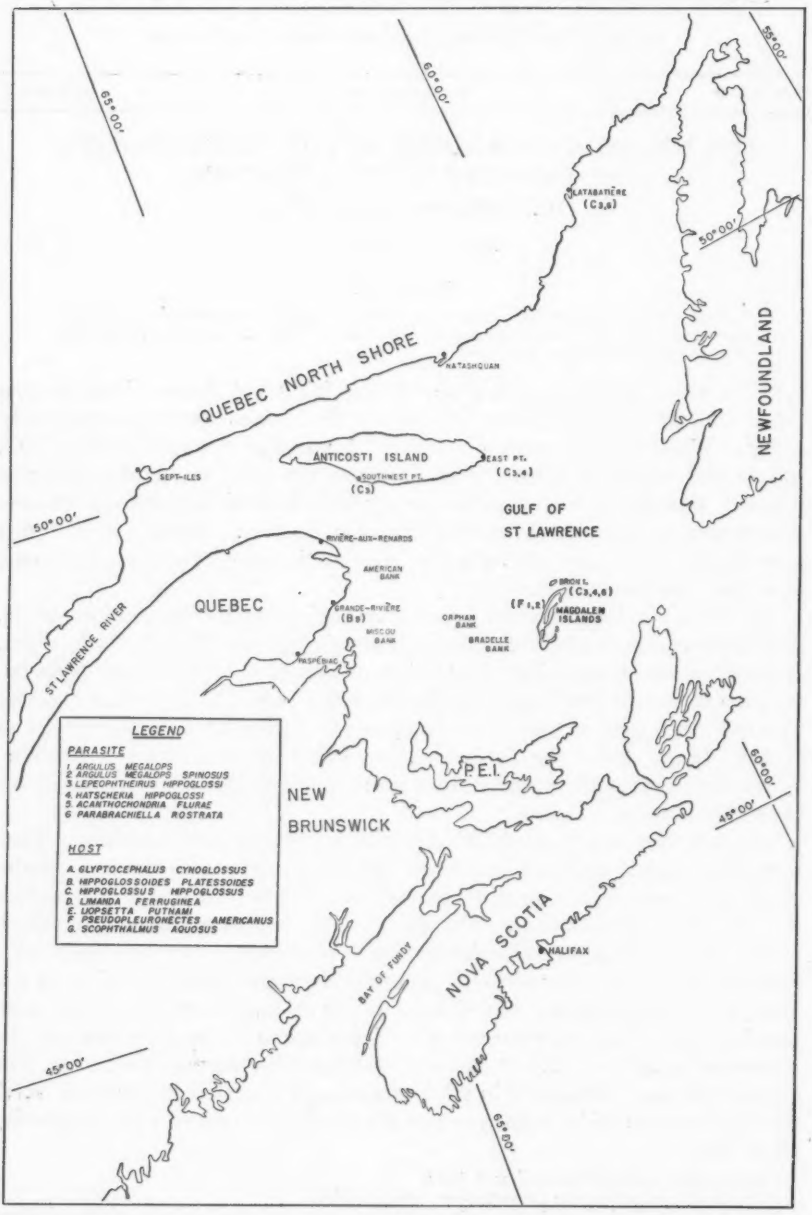

FIG. 1. The distribution of the copepod parasites of the Heterosomata in the Gulf of St. Lawrence.

Order Copepoda

The parasites of the order Copepoda were classified according to Sar's comprehensive work, with those modifications thought requisite by Wilson (7).

Suborder ARGULOIDA
Family ARGULIDAE
Genus *Argulus* Müller, 1785

Argulus megalops Smith, 1872–73

Host: Pseudopleuronectes americanus.
Location: Skin, over entire body surface.
Locality: Magdalen Islands.

The host specimens were taken from House Harbour lagoon by means of a small plankton beam trawl. The water of this lagoon is shallow, and therefore of a higher temperature during the summer months than the surrounding ocean. In a sample of 15 fish examined, it was found that they all carried this parasite, but there were never more than two specimens present; all parasites were female. The identification was made by comparing the specimens with Wilson's (7, 8) and Meehean's (4) descriptions of *A. megalops*. The presence of nine saucer-shaped segments and one rectangular basal segment in each supporting rod of the maxillary sucking disk was confirmation of the diagnosis. This parasite has been found previously on the winter flounder (*P. americanus*), summer flounder (*Paralichthys dentatus*), sand flounder (*Scophthalmus aquosus*), plaice (*H. platessoides*), sea robin (*Prionotus carolinus*), long horned sculpin (*Myoxocephalus octodecimspinosus*), and goosefish (*Lophius piscatorius*). All of these hosts are part of the littoral benthos.

Argulus megalops spinosus Wilson, 1944

Host: Pseudopleuronectes americanus.
Location: Skin, over entire body surface.
Locality: Magdalen Islands.

These parasites were taken from the same 15 fish noted above as carrying *Argulus megalops*. The infection by the subspecies was heavier, in that up to 37 specimens were taken from one winter flounder only 75 mm. in length. Previous collections of this parasite have been made by Johansen; these were identified by Wilson (8). He gave the hosts as *Liopsetta putnami* and *Acanthocottus octodecimspinosus*. A few specimens of *L. putnami* were examined from the same area but never in the live state, so that it was impossible to substantiate Johansen's findings for the Gulf. The morphology of the subspecies does not differ greatly from that of *A. megalops*. The antennae and mouth parts of the female differ slightly; in both sexes the presence of five or six saucer-shaped segments and one longer basal segment in the maxillary sucking disk are the main distinctive characteristics. The ratio of males to females was found to be in the order 2:5.

Suborder CALIGOIDA
Genus *Lepeophtheirus* Nordmann, 1832

Lepeophtheirus hippoglossi (Krøyer, 1837)

Host: Hippoglossus hippoglossus.
Location: Skin, usually near the head on the dorsal surface.
Locality: East and South West Point, Anticosti Island; Brion Island, Magdalen Islands; LaTabatière, north shore of the Gulf of the St. Lawrence.

The two specimens from East Point, Anticosti Island, were taken from a medium sized halibut. One of these copepods was a male 12 mm. in length, the other a female 14 mm. in length. These two specimens were larger than those described by Scott, T. and A. (5), and the sexual dimorphism was less marked.

In April of 1956, two halibut were taken on a long line in 9 meters of water off South West Point, Anticosti Island. These two fish together carried 46 specimens of *Lepeophtheirus hippoglossi*, 32 of which were females. In the case of 11 of the 14 males, the hyperparasitic monogenetic trematode *Udonella caligorum* was present; the female of the copepod species showed a lower incidence of parasitic infection, only five of the female *L. hippoglossi* carried *U. caligorum*. This latter parasite will be discussed more fully under the description of the trematodes recorded in this survey.

The specimens from the Brion Island halibut were part of a composite sample of ectoparasites from 12 fish, captured on hand lines in September of 1954. The total number of parasites was 29, of which 28 were females; the solitary male was found in the company of a female on one host. These specimens fitted more closely to the length requirements given by Scott, T. and A. (5). The mean length of the 28 females was 13.0 mm., while the male measured 8.0 mm. in length.

A halibut, captured in the summer of 1953 at LaTabatière, was found to be parasitized by 20 *L. hippoglossi* females, the mean length of which was closer to those of Brion Island specimens than the Anticosti material.

Family DICHELESTRIIDAE
Genus *Hatschekia* Poche, 1902

Hatschekia hippoglossi (Krøyer, 1837)

Host: Hippoglossus hippoglossus.
Location: Gills.
Locality: Brion Island, Magdalen Islands; East Point, Anticosti Island.

A small halibut, 22.5 kg. in weight, taken in 1954 at Brion Island, carried three females of *Hatschekia hippoglossi*. A larger halibut, taken later in the same year, was more heavily parasitized with 12 copepods, of which one was a male. Another halibut, taken at the same time, was found to be carrying eight females of this parasite in the branchial cavity.

Both fish from the Anticosti area had 10 *H. hippoglossi* each in their gills; one of these was a young specimen, in which the distal ends of the second pair of antennae had not been formed into apical hooks. All specimens were females.

The living specimens were flat, white in color, the ovisacs a reddish-gray, the intestine a dark line within the body cavity.

The length of all the specimens taken lay between 7 and 9 mm. This agrees closely with Wilson (7), who gave the length as 6 to 8 mm., and Scotts' (5) figure of 9 mm. The egg strings were 10 mm. long on the largest female, again a substantiation of Krøyer's (3) and Wilson's (7) figures.

The apical hook of the second antenna is simple, not barbed and heavily sclerotized. The length from its base, at the distal end of the antenna to the furthest extremity of the recurved hook, is 0.28 mm.

Suborder LERNAEOPODOIDA
Family CHONDRACANTHIDAE
Genus *Acanthochondria* Oakley, 1927

Acanthochondria flurae (Krøyer, 1863)

Host: Hippoglossoides platessoides.
Location: Branchial and oral cavities.
Locality: Grande-Rivière.

A medium sized fish taken in May, 1955 was parasitized by one specimen of *A. flurae.* The site of attachment was on the inner dorsal surface of the mouth. Two further specimens were taken in 1956, from two plaice measuring approximately 30 and 37 cm. in length respectively. On one fish, the site of infection was the first gill ray, while on the other, the copepod was firmly attached to the inside of the operculum. In the case of the last two fish, the examination was made immediately upon bringing the fish on board the boat, so that the position of infection was certain. The situation of the parasite in the previous case was unusual, unless the copepod had moved from its favored site in the gills, upon the death of the host. All of the specimens examined were female. The hosts were taken close to the shore in 9 to 18 meters of water.

Family LERNAEOPODIDAE
Genus *Parabrachiella* Wilson, 1915

Parabrachiella rostrata (Krøyer, 1837)

Host: Hippoglossus hippoglossus.
Location: Gills.
Locality: Brion Island, Magdalen Islands; LaTabatière, north shore of the Gulf of St. Lawrence.

The Brion Island halibut had six females of this species in the gills, the Gulf north shore halibut three females. The male was not found. The lengths of the copepods were shorter than those previously recorded by Wilson

(7), who gave the measurement as 5 to 6 mm. for the length of the cephalothorax and 6 to 6.5 mm. for the length of the trunk. In the present material, the cephalothorax was 4 to 5.5 mm., the trunk 4 to 5 mm. in length.

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ASPECTS QUANTITATIFS DES BESOINS EN MINÉRAUX DE TRIBOLIUM CONFUSUM DUVAL

II. POURCENTAGE OPTIMUM DES CATIONS Mg, Ca, Na et K[1]

L. HUOT,[2] R. BERNARD ET A. LEMONDE[3]

Abstract

The quantitative requirements of the *Tribolium confusum* larvae for magnesium, calcium, sodium, and potassium are reported. Magnesium and potassium are definitely essential for larval growth. Without these elements, the larvae do not survive long. Calcium appears essential for imaginal metamorphosis. In its absence, rapid dehydration of the pupae occurs and emergence of the adult is practically suppressed. The importance of sodium could not be demonstrated because the minute quantities already present in the basal diet appear sufficient to meet the requirements of this insect. The requirements of both sexes for these cations are of the same order of magnitude. Additions of these cations in excess of the optimum values are well tolerated.

Introduction

Dans un travail antérieur, (4) nous avons recherché le pourcentage optimum du mélange salin no 2 U.S.P. XIII qu'il faut inclure dans un régime pour que les larves de *Tribolium confusum* croissent et se métamorphosent normalement. Les résultats que nous avons obtenus démontrent que cette espèce d'insecte exige la présence de 1 à 8% du mélange salin no 2 pour compléter sa croissance larvaire et de 2 à 8% de ce mélange pour accomplir sa métamorphose imaginale.

Nous basant sur ces premières données nous avons déterminé dans quelle proportion les larves mâles et femelles de *T. confusum* ont besoin de magnésium, de calcium, de sodium, et de potassium pour atteindre le stade adulte.

Méthodes expérimentales

Toutes les méthodes expérimentales utilisées au cours de ce travail ont été décrites antérieurement (4). La composition du régime de base et celle du mélange salin no 2 sont exposées dans les tableaux I et II. Au régime de base, nous avons ajouté 4% du mélange salin, ce pourcentage étant intermédiaire entre les taux minimum et maximum tolérés par l'insecte.

Pour rechercher le pourcentage optimum des cations Mg, Ca, Na et K, nous avons varié tour à tour chacun de ces cations, en ayant soin de maintenir constant le taux des autres ions du mélange salin. Le régime était toujours porté à 100 g. par l'addition de sucrose.

Nous avons tenu compte du sexe de tous les individus mis en expérience. Nous voulions ainsi nous rendre compte si l'utilisation de l'un ou l'autre des cations pouvait varier en fonction du sexe.

[1]Manuscrit reçu le 11 septembre 1957.
Contribution du département de Biochimie de la Faculté de Médecine et du département de Biologie de la Faculté des Sciences, Université Laval, Québec, faite avec l'aide financière du Conseil des Recherches Agricoles, Ministère de l'Agriculture, Québec.

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TABLEAU I

COMPOSITION DU RÉGIME DE BASE UTILISÉ

Principaux ingrédients	%	Vitamines	μg. par g. de régime
Caséine*	20.0	Chlorhydrate de thiamine	1
Cholestérol	1.0	Riboflavine	2
		Chlorhydrate de pyridoxine	1
Sucrose	79.0	Acide nicotinique	8
		Pantothénate de calcium	4
		Choline	1500
		Biotine	0.05
		Acide folique	0.2

*"Vitamin free" casein— Nutritional Biochemicals Corporation, Cleveland, Ohio.
Ions inorganiques présents dans cette caséine: sodium 0.02%, calcium 0.03%, phosphore 0.79%, chlorures 0.1%, fer 13 p.p.m., potassium 0.

TABLEAU II

COMPOSITION DU MÉLANGE SALIN NO 2 U.S.P. XIII

Sels	Formules chimiques	G. par 100 g. de mélange	
		Sel total	Cation
Orthophosphate monocalcique	$Ca(H_2PO_4)_2.H_2O$	13.58	2.16
Lactate de calcium	$Ca(C_3H_5O_3)_2.5H_2O$	32.70	4.25
Citrate ferrique	$FeC_6H_5O_7.5H_2O$	2.97	0.495
Sulfate de magnésium	$MgSO_4.7H_2O$	13.70	1.35
Orthophosphate dipotassique	K_2HPO_4	23.98	10.76
Orthophosphate monosodique	$NaH_2PO_4.H_2O$	8.72	1.45
Chlorure de sodium	NaCl	4.35	1.71

Résultats et discussion

Besoins en magnésium

Les résultats que nous avons groupés dans le tableau III démontrent nettement l'importance du magnésium dans la nutrition des larves de *T. confusum*. En effet, dans un régime pauvre en cet élément, les larves ne croissent pas et meurent très tôt. Déjà, l'addition de 5 mg. de magnésium par 100 g. de régime permet à la moitié des larves mises en expérience d'atteindre le stade pupe et la métamorphose imaginale. La durée de la période larvaire est alors de 54.9 jours.

La quantité optimum de magnésium requise par nos larves se situe entre 50 et 200 mg. par 100 g. de régime. Avec des quantités de cet ordre, la vie larvaire dure en moyenne 35 ou 36 jours et la majorité des larves deviennent adultes.

T. confusum manifeste une grande tolérance vis-à-vis un excès de sulfate de magnésium. En effet, pour affecter véritablement la croissance et la survie des larves, il faut ajouter à la diète près de 10% de ce sel. A ce taux, 15 larves sur 30 ont atteint le stade pupe en 57.5 jours. Nous avons vu, dans

une publication précédente (4), qu'un régime contenant 20% de mélange salin no 2 retarde la croissance d'environ 20 jours. Ce retard n'est sûrement pas dû au sulfate de magnésium.

Le magnésium ne semble pas jouer un rôle prépondérant dans la métamorphose imaginale. En général, quelle que soit la quantité de ce cation dans le régime, toutes les larves qui parviennent à puper accomplissent très bien leur métamorphose en adultes.

L'importance de cet élément a été étudié chez plusieurs autres espèces d'insectes. Ainsi Sang (7) a observé que le magnésium est essentiel à *Drosophila melanogaster*. Chez les larves de *Theobaldia incidens*, une carence en magnésium retarde la croissance (2). En 1939, Bates (1) a prouvé que la présence de sels de magnésium dans la diète favorise la survie des larves de plusieurs espèces d'*Anopheles*. Par contre, Trager (8) a démontré que chez *Aëdes aegypti* le sulfate de magnésium n'est pas essentiel.

TABLEAU III

IMPORTANCE DU SULFATE DE MAGNÉSIUM DANS LA NUTRITION DES LARVES DE *Tribolium confusum*

Sel de magnésium inclus dans 100 g. de régime		Nombre total de pupes sur 30 larves		Nombre de jours requis pour atteindre le stade pupe		Nombre total d'adultes sur 30 larves		Nombre de jours requis pour atteindre le stade adulte	
$MgSO_4.7\ H_2O$ (g.)	Mg^{++} (mg.)	♂	♀	Moyenne $\pm\sigma_M$	Temps limites	♂	♀	Moyenne $\pm\sigma_M$	Temps limites
0.00	0	0	0	–	–	0	0	–	–
0.05	5	7	8	54.9 ± 7.97	36–39	7	7	63.6 ± 2.16	49–76
0.10	10	9	14	40.3 ± 0.97	30–50	9	13	46.9 ± 0.96	36–57
0.20	20	13	11	38.1 ± 0.72	31–46	13	10	45.0 ± 0.79	37–54
0.30	30	12	15	38.3 ± 0.48	31–42	11	12	45.4 ± 0.43	41–49
0.40	40	13	12	37.7 ± 0.69	31–43	13	12	43.9 ± 0.79	37–49
0.50	50	18	7	35.3 ± 0.60	30–40	14	7	42.4 ± 0.79	34–48
0.60	60	7	15	35.2 ± 0.66	30–40	7	14	41.7 ± 0.73	35–47
2.00	200	12	13	35.9 ± 0.49	30–42	12	13	43.3 ± 0.51	35–50
4.00	400	7	15	41.7 ± 0.93	33–49	5	15	48.9 ± 0.94	41–56
6.00	600	15	6	48.2 ± 1.53	35–61	15	6	55.6 ± 1.48	42–68
10.00	1000	7	8	57.5 ± 1.80	46–69	7	8	65.5 ± 1.55	58–76

Besoins en calcium

Nous réunissons dans le tableau IV les résultats que nous avons obtenus dans la recherche des besoins en calcium des larves de *T. confusum*. Il faut noter ici que nous avons toujours ajouté les deux sels de calcium, mentionnés dans le tableau II, dans des proportions identiques à celles qui existent dans le mélange salin no 2.

Lorsque nous enlevons complètement le calcium du mélange salin, il reste dans la diète environ 6 mg. de calcium par 100 g., soit la quantité apportée par la caséine utilisée. Les larves atteignent alors le stade pupe après un retard d'environ 10 jours sur la durée normale, qui est voisine de 35 jours. Les pupes qui proviennent de ces larves sont petites, et les ailes imaginales, que nous apercevons dans le ptérothèque, présentent souvent une teinte brunâtre à leurs extrémités. Dès les premières heures de la transformation, les individus se déshydratent lentement. La teinte brunâtre envahit les différentes parties de la bouche et les pattes, qui sont enveloppées par les

podothèques. Finalement, les pupes entières deviennent brunes et même parfois noires. En quelques jours, elles se dessèchent complètement et aucune émergence n'est possible. Pour prévenir cette déshydratation, il faut ajouter au moins 124 mg. de calcium par 100 g. de régime.

Dans notre étude (4) sur le pourcentage optimum du mélange salin no 2 nous avions observé que, dans les régimes contenant de petites quantités de sels, soit 0.5% à 1.5%, les pupes semblaient éprouver de la difficulté à compléter leur métamorphose imaginale. Les larves qui croissaient dans ces régimes pauvres en mélange salin donnaient des pupes présentant les mêmes signes de déshydratation. À la lumière des présents résultats, une carence en calcium nous apparaît comme étant la cause de cette anomalie.

Pour obtenir une croissance optimum, il faut environ 290 mg. de calcium par 100 g. de régime, soit à peu près 4% du mélange salin no 2. Si nous portons à 1% le calcium du régime, la durée moyenne du stade larvaire augmente sensiblement; avec un taux de 3.5%, la croissance cesse complètement. La tolérance des larves de *T. confusum* vis-à-vis un excès de calcium est donc plus grande que pour le magnésium.

TABLEAU IV

IMPORTANCE DES SELS DE CALCIUM DANS LA NUTRITION DES LARVES DE *Tribolium confusum*

Sels de calcium inclus dans 100 g. de régime		Nombre total de pupes sur 30 larves		Nombre de jours requis pour atteindre le stade pupe		Nombre total d'adultes sur 30 larves		Nombre de jours requis pour atteindre le stade adulte	
$Ca(H_2PO_4)_2.H_2O$ et $Ca(C_4H_5O_3)_2.5H_2O$ (g.)	Ca^{++} (mg.)	♂	♀	Moyenne $\pm \sigma_M$	Temps limites	♂	♀	Moyenne $\pm \sigma_M$	Temps limites
0.0	0	12	8	44.4 ± 1.19	37–55	1	0		56 ème
0.1	14	12	12	41.5 ± 0.63	36–47	3	2	48.6 ± 1.18	45–51
0.5	69	10	15	37.4 ± 0.59	31–44	6	10	44.3 ± 0.63	40–50
0.9	124	14	13	37.9 ± 0.71	30–46	11	10	48.9 ± 0.65	40–51
1.3	180	13	12	38.3 ± 0.63	31–45	13	10	45.0 ± 0.65	37–51
2.1	291	10	16	34.2 ± 0.58	30–39	10	14	40.8 ± 0.80	36–46
5.0	683	10	12	35.1 ± 0.57	30–40	10	12	41.8 ± 0.62	36–46
10.0	1385	12	11	38.9 ± 0.48	33–47	12	11	46.0 ± 0.50	39–53
20.0	2770	5	7	49.8 ± 1.55	43–58	4	5	57.1 ± 1.52	50–64
25.0	3463	0	1	–	58 ème	0	1	–	63 ème

On sait que plusieurs espèces d'insectes exigent la présence de calcium dans leur alimentation. Les travaux de Trager (8) ont montré les effets très favorables de cet élément sur *Aëdes aegypti*. Frost, Herms et Hoskins (2) ont enregistré des résultats semblables chez les larves de *Theobaldia incidens*. Bates (1) a observé que toutes les espèces d'*Anopheles* qu'il a étudiées ont besoin de calcium dans leur régime. Par contre, les larves de *Drosophila melanogaster* peuvent se passer de cet élément pour croître et se métamorphoser normalement. Loeb (5), puis Rubinstein, Lwowa et Buriakowa (6) ont observé d'abord ces résultats et récemment Sang (7) les a confirmés.

Besoins en sodium

Les données inscrites dans le tableau V semblent prouver que les larves de *T. confusum* peuvent croître et se métamorphoser normalement sans le concours d'un apport de sodium. En effet, quand on a exclu cet élément du

mélange salin, 24 larves ont atteint le stade pupe en 39.5 jours. Il faut noter cependant que le régime n'était pas complètement dépourvu de sodium, puisque la caséine utilisée en fournit 4 mg. par 100 g. de régime.

Si nous ajoutons diverses quantités de sodium au régime, en observant toujours entre les deux sels de sodium les mêmes proportions que dans le mélange salin no 2, la croissance est à peu près la même. En effet, il n'existe aucune différence significative entre les moyennes qui apparaissent au tableau V. Si la teneur en sels de sodium dépasse 3%, les régimes changent de texture. Ils prennent un aspect gommeux et granuleux, ce qui nuit considérablement au développement larvaire. La mortalité élevée dans un tel régime peut être due aussi bien à la condition physique qu'à la toxicité des sels de sodium.

Notre Ténébrionide semble se comporter comme *Aëdes aegypti.* Trager (8) a démontré en effet que ce Diptère peut se développer normalement en l'absence de sodium. Par contre, la présence de cet élément est indispensable à la croissance chez *Drosophila melanogaster* (7), *Theobaldia incidens* (2) et plusieurs espèces d'*Anopheles* (1).

TABLEAU V

IMPORTANCE DES SELS DE SODIUM DANS LA NUTRITION DES LARVES DE *Tribolium confusum*

Sels de sodium inclus dans 100 g. de régime		Nombre total de pupes sur 30 larves		Nombre de jours requis pour atteindre le stade pupe		Nombre total d'adultes sur 30 larves		Nombre de jours requis pour atteindre le stade adulte	
NaCl et NaH_2PO_4 (g.)	Na^+ (mg.)	♂	♀	Moyenne $\pm \sigma_M$	Temps limites	♂	♀	Moyenne $\pm \sigma_M$	Temps limites
0.0	0	11	13	39.5 ± 0.79	34–48	11	11	46.2 ± 0.83	41–55
0.5	120	10	10	36.9 ± 0.88	30–44	10	9	43.5 − 0.92	36–51
1.0	240	11	12	37.0 ± 0.56	31–41	10	10	43.6 − 0.59	38–47
3.0	730	10	10	39.1 ± 0.68	31–47	10	9	46.3 − 0.82	37–56
6.0	1450	0	0	–	–	0	0	–	–
10.0	2420	0	0	–	–	0	0	–	–

L'importance des chlorures dans l'organisme animal est établie depuis longtemps, et leur principale source dans l'alimentation est généralement le chlorure de sodium. Voilà pourquoi on ne peut guère distinguer les besoins en chlorures des besoins en sodium. Cependant, même si le régime est dépourvu de chlorure de sodium, il existe encore du chlore assimilable dans la caséine, la choline et quelques autres vitamines. Ces différentes sources chlorurées fournissent approximativement 20 mg. de chlore par 100 g. de régime. Cette quantité serait donc suffisante pour subvenir aux exigences de *T. confusum.*

De ces résultats, nous pouvons conclure que l'omission des sels de sodium et des chlorures dans le mélange salin no 2 n'est aucunement préjudiciable à la croissance larvaire de *T. confusum.* Cela ne signifie pas nécessairement qu'il faille éliminer complètement le chlorure de sodium du mélange salin. Nous croyons plutôt que la présence de ce sel, même en faibles quantités, peut avoir un effet favorable sur la croissance. En tous cas, avant de pouvoir conclure dans un sens ou dans l'autre, il faudrait supprimer toute source possible de chlore et de sodium dans le régime, pour éviter la transmission de toute trace de ces éléments aux embryons.

Besoins en potassium

Les résultats colligés dans le tableau VI nous permettent d'attribuer une grande importance à l'ion potassium dans la nutrition des larves de *T. confusum*.

Au cours de cette dernière expérience, la durée du stade larvaire a été plus longue et moins de larves ont atteint le stade pupe. Nous croyons que cette anomalie est due aux difficultés que nous avons rencontrées avec l'étuve à insectes au cours de notre étude sur le potassium. Nous avons conservé quand même ces résultats parce qu'ils montrent le rôle essentiel du potassium. D'ailleurs, nous pouvons très bien comparer ces résultats avec ceux des autres expériences; les moyennes sont plus élevées mais, les erreurs standard (σ_M) étant plus grandes, les différences deviennent donc moins importantes.

TABLEAU VI

IMPORTANCE DU POTASSIUM DANS LA NUTRITION DES LARVES DE *Tribolium confusum*

Sel de potassium inclus dans 100 g. de régime		Nombre total de pupes sur 30 larves		Nombre de jours requis pour atteindre le stade pupe		Nombre total d'adultes sur 30 larves		Nombre de jours requis pour atteindre le stade adulte	
K_2HPO_4 (g.)	K^+ (mg.)	♂	♀	Moyenne $\pm\sigma_M$	Temps limites	♂	♀	Moyenne $\pm\sigma_M$	Temps limites
0.00	0	0	0	–	–	0	0	–	–
0.02	10	0	0	–	–	0	0	–	–
0.06	30	0	2	67.0 ± 4.01	63–71	0	2	73.5 ± 2.56	71–76
0.10	50	7	6	64.9 ± 2.86	51–82	6	5	71.6 ± 2.58	59–88
0.14	60	7	6	54.6 ± 3.17	38–82	6	5	62.7 ± 3.49	46–88
0.20	90	10	6	48.8 ± 2.40	37–57	9	5	56.6 ± 2.64	44–81
0.30	140	6	14	43.7 ± 1.25	35–56	6	10	50.9 ± 1.29	42–62
0.60	270	11	11	41.4 ± 1.48	31–55	7	11	48.2 ± 1.62	39–62
1.00	450	14	11	37.0 ± 0.70	30–43	14	11	44.5 ± 0.74	36–50
3.00	1350	11	9	40.7 ± 1.09	32–52	8	9	48.5 ± 1.24	40–60
5.00	2240	14	11	42.2 ± 1.16	36–55	9	11	51.0 ± 1.23	44–62
10.00	4490	0	0	–	–	0	0	–	–

Pour obtenir une croissance optimum, il faut inclure à peu près 450 mg. de l'ion potassium pour 100 g. de régime. Avec une quantité inférieure à 50 mg. pour 100 g. de régime, quelques larves seulement parviennent au stade pupe. C'est dire que le potassium est tout à fait indispensable à la croissance et à la métamorphose de *T. confusum*. Par contre, de trop grandes quantités de potassium agissent comme le sodium et confèrent à la diète une consistance gommeuse, qui rend impossible toute croissance larvaire.

Plusieurs autres espèces d'insectes exigent la présence de potassium dans leur alimentation. Ainsi Sang (7) l'a démontré chez *Drosophila melanogaster*. Chez certaines espèces d'*Anopheles*, le pourcentage de survie est augmenté en présence de potassium (1). Un défaut de chlorure de potassium entraîne un retard dans la croissance de *Theobaldia incidens* (2). Un récent travail de House et Barlow (3) révèle l'importance de cet élément dans la nutrition de *Pseudosarcophaga affinis*. Certaines espèces peuvent cependant se passer entièrement du potassium. Trager (8) a observé que les larves d'*Aëdes aegypti*, par exemple, peuvent croître normalement dans un régime dépourvu de cet élément.

Pour tous les essais, avec chacun des cations étudiés, nous avons toujours tenu compte du sexe des individus. Nous n'avons pu déceler aucune différence significative entre les moyennes calculées pour les deux sexes. Les besoins en ces quatre cations sont donc identiques pour les mâles et les femelles.

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STUDIES ON THE DEVELOPMENT OF THE KIDNEY OF THE PACIFIC PINK SALMON, (ONCORHYNCHUS GORBUSCHA (WALBAUM))

I. THE DEVELOPMENT OF THE PRONEPHROS[1]

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Abstract

The literature relating to pronephros development in fishes is discussed and the course of development in the pink salmon described. The vascularization of the kidney is described and the histological differentiation of the elements of the kidney listed with special respect to the epithelia of the tubules. The appearance of the myeloid tissue is noted.

Introduction

This study was undertaken as a contribution to the understanding of teleost kidney development. In addition, it was hoped that a study of the kidney of a migratory fish might aid in the interpretation of the osmoregulatory role of the teleost kidney. The development of the pronephros and the development of the mesonephros will be separately presented.

The hypothesis upheld by Felix (6) that pro-, meso-, and meta-nephroi are three distinct sets of organs laid down in chronological succession along the trunk is no longer accepted (5). Balfour and Sedgwick (1) interpret the three as regions of a single primitively metameric organ. Descriptive work on lower craniates (*Hypogeophis* (3), *Bdellostoma* (13, 14), and more recent experimental studies on the chick (2, 9, 10) and Amphibia (5)) have confirmed the homology of the three kidneys. Price (13, 14) advanced the name holonephros to describe the serial homologous pro- and meso-nephroi of *Bdellostoma*. Fraser (8) recommends application of the term to the entire vertebrate kidney. She recommends retention of the older terminology for convenience of description.

Primitively, the narrow cavity between the somatic and splanchnic layers of the intermediate cell mass is continuous ventrally with the splanchnocoele and dorsally with the myocoele. The area nearest the somite dilates to form a conspicuous coelomic chamber, or nephrotome (15). The connection with the splanchnocoele is retained longer than that with the myocoele but is early reduced to a narrow peritoneal funnel by ingrowth of cells from either end of the nephrotome. A solid outgrowth from the dorsal wall of each nephrotome later becomes a uriniferous tubule which opens to the dilated nephrotome, now called a pronephric chamber, by way of a nephrostome. Distally the uriniferous tubule becomes connected with the duct. Fraser (8) recommends the name segmental duct to emphasize its primitively metameric origin.

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Cells migrate from the medial surfaces of the intermediate cell masses and from the nephrotomes at a later stage along the entire length of the trunk. These cells form a darkly staining mass, which Maschkowzeff (11) called the venous strand, immediately beneath the hypochord. Since it is already established that this mass of cells gives rise not only to the cardinal veins but also to the aorta, glomeruli, and certain skeletal elements (Fraser (8)), the name vascular strand ("masse vasculaire") used by Swaen and Brachet (19) seems more appropriate. The medial wall of the pronephric chamber is subsequently invaded by a vascular tuft, the glomerulus, which may be derived from an aortic shoot (19), or may develop in situ and only later connect to the aorta (Balfour and Sedgwick (1); D. and J. Davies, unpublished results in Fraser (8)). The postglomerular blood is returned to the heart.

The teleost pronephros develops from the intermediate cell mass of several segments. A single tubule on either side opens into a thin-walled elongated pronephric chamber which may extend over one to three segments. The chamber is originally in connection with the coelom but soon separates from it. Separate glomeruli project into either chamber. Fusion of the right and left glomeruli forms a single large median glomerulus which extends the full length of the pronephric chambers. Distally, the tubules enter the right and left segmental ducts which pass posteriad to unite anterior to the cloaca. Rudimentary pronephric structures appear in a few segments anterior and posterior to the definitive pronephros (Brachet (4)). In the mesonephric region salmonids commonly possess only a single median cardinal vein continuous with the caudal vein. Posterior to the pronephros the median cardinal vein branches into right and left cardinal sinuses which surround the pronephric tubules to form an embryonic portal system (Shearer (17)).

Materials and Methods

Pink salmon eggs were collected at Cultus Lake in November, 1953, and artificially fertilized before transportation to the university hatchery. They were kept in fresh water tanks at temperatures which ranged from 12.5° in November, through 5° C. in January, to 15° in August. Hatching began on the 50th day after fertilization. The fry were fed on a mixture of canned salmon and Pablum. Samples of the early stages were taken every hour. Thereafter the intervals were gradually increased to 2 weeks for the latest stages sampled. The fresh water series was continued until August, 1954. Migrating fry were trapped in April, 1955, as they entered the sea at Port John, B.C., and held in salt water pens in the bay. These specimens were sampled weekly at first, but later bimonthly until August, 1955. Further sea water specimens were taken during their migration into the open Pacific in July and August of 1955.

The fry were fixed whole in Smith's formal bichromate or Bouin's fluid. Smith's was found more satisfactory for the earlier yolky stages. Fry collected in the field were fixed in Bouin for convenience.

Sections were cut at 10 μ.

Heidenhain iron haematoxylin, with or without a counterstain, was found satisfactory.

Description of Embryos

The pronephros develops from the segmented intermediate mesoderm of the second to the 10th segments. The intermediate cell mass appears as a triangular block of loosely packed cells lateral to the somites and medial to the lateral plate in an embryo of 2.15 mm. (eight somites). The mass is distinctly separated from the somites but continuous with the lateral plate (Fig. 1A). This condition is transitory in the pronephric region but persists longer in the mesonephric region. Segmental pronephric rudiments later appear in the second to the fifth segments. The intermediate cell masses of the sixth and seventh segments, from which the definitive pronephros develops, are fused together as are those posterior to the 10th somite. No nephrotomes appear posterior to the seventh segment.

By 3.33 mm. (18 somites) the coelom extends as far as the fifth segment. The rudimentary nephrocoeles of the second to the fourth segments are widely open to the coelom. The nephrotomes and pronephric folds appear only as segmental thickenings of the dorsal and medial walls of the splanchnocoele. The rudimentary fifth nephrotome is still apparent (Fig. 1B). In the sixth and seventh segments the lateral plate and nephrotomes are continuous and are clearly divided into somatic and splanchnic layers but no coelomic cavity appears. From the fused nephrotomes a single solid pronephric fold (tubule rudiment) grows dorsolaterally to press against the ectoderm.

By the time the embryo is 3.45 mm. long the nephrotomes of the second to the fourth segments are entirely incorporated in the coelomic wall. In the fifth, the pronephric fold remains as a thickened area of the dorsal coelomic wall. The somatic and splanchnic layers of the lateral plate and fused nephrotomes are widely separated in the sixth and seventh segments by the coelomic cavity. Nephrocoele and splanchnocoele are continuous over their entire length. In the more anterior region of the sixth segment the nephrocoele is widely open to the splanchnocoele (Fig. 1C). More posteriorly, ventromedial extension of the splanchnocoele has begun. The consequent rotation of the pronephric rudiment considerably narrows the opening between nephrocoele and splanchnocoele (Fig. 1D).

Posterior to the seventh segment a solid rod of cells (duct rudiment) continuous with the pronephric fold of the seventh segment becomes separated from the remainder of the intermediate cell mass. In the 3.33 mm. embryo, it is apparent in the eighth to the 10th segments. As segmentation and elongation of the embryo proceed the rod is added to in each segment until it reaches the 39th segment (4.4 mm. embryo). Tubulation of the rod in the anterior segments begins when the separation of material has proceeded as far as the 18th segment (3.5 mm.). From the 39th segment its tip grows ventrad to unite with that of the other side. The combined ducts enter the cloaca at the level of the 40th segment just behind the gut. The segmental duct is fully formed by the time the embryo is 5.24 mm. long (Fig. 2G).

Continued extension of the splanchnocoele beneath the fused nephrotomes of the sixth and seventh segments rotates the pronephric rudiment so that the connection to the splanchnocoele becomes ventrally directed (3.45–4.4 mm.) (Fig. 1E). The connection between the splanchnocoele and the nephrocoele becomes narrowed until in cross section it has the appearance of a peritoneal funnel and short peritoneal canal. Since the opening extends the entire length of the nephrotome it cannot be regarded as a pronephric funnel. Distinct peritoneal funnels do not form at any stage. In the 4.4 mm. embryo the nephrocoele extends into the pronephric fold and is continuous with the lumen of the developing duct.

Between 3.3 and 4.05 mm. rotation of the rudiment and ingrowth of cells from either end of the cavity closes the connection between the fused sixth and seventh nephrocoeles and the splanchnocoele. A similar process leads to the formation of a short narrow tubule which connects the fused nephrocoeles to the duct. The walls of nephrotome, tubule, and duct are of uniform tall columnar cells. In the 3.33 mm. embryo, migration of cells from the medial surface of the intermediate cell mass and of the nephrotomes has established the vascular strand as a mass of loosely packed cells beneath the hypochord. In the most anterior segments the dorsal aorta is differentiated as a fine endothelial tube within the mass (Fig. 1B).

In subsequent stages, medial extension of the combined sixth and seventh nephrocoeles results in enlargement of the cavity and thinning of the epithelium from columnar to cuboidal. The cavity becomes a recognizable pronephric chamber (Fig. 1F). The chambers of the two sides are at first separated in the mid-line by the mass of vascular strand tissue. In most specimens differentiation of the cardinal veins is not observed until the 4.4 mm. stage. A capillary vessel, which also develops in situ in the vascular strand, invaginates the entire length of the thin medial wall of each pronephric chamber. The glomeruli so formed (Fig. 1F) become secondarily connected to the aorta. The postglomerular blood enters the postcardinal sinuses which surround the uriniferous tubule. After a single anterior loop the uriniferous tubule passes posteriad to enter the duct. Blood from the postcardinal vein enters the right and left postcardinal sinuses and so establishes an embryonic renal portal system. The system persists only as long as the pronephros is functional. The left postcardinal does not develop posterior to the pronephros.

Histological differentiation of the pronephric elements begins by 4.76 mm. Simple squamous epithelium characterizes the wall of the pronephric chamber, except at the nephrostome where it is transitional between simple squamous and the tall simple columnar epithelium of the neck segment. Cells of the neck segment are laterally compressed. There is an abrupt change to the tall columnar weakly acidophilic cells of the tubule. The tubule cells are later characterized by their distinct brush border (10 mm.). The epithelium changes abruptly to the low columnar cells typical of the duct. The glomeruli of the two sides and their arterial connections fuse completely. Dorsal and ventral to the fused glomeruli, the median walls of the pronephric chambers

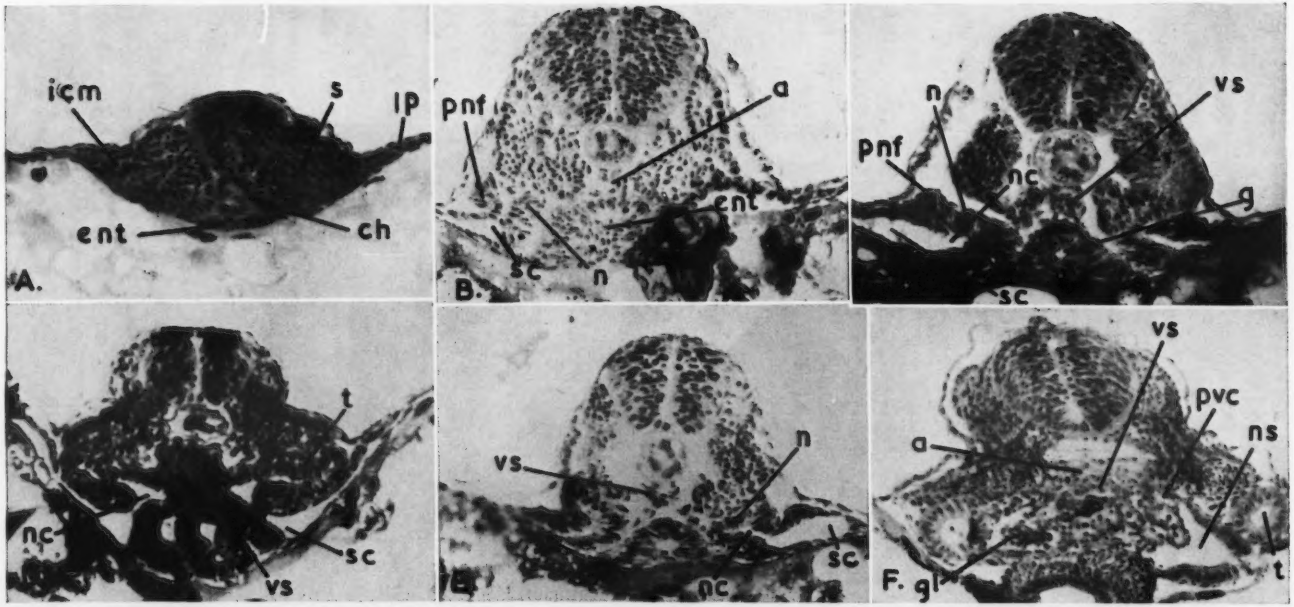

FIG. 1. A. The differentiation of mesodermal elements; T.S. 2.15 mm. embryo (eight somites) through the fifth somite; × 200. B. Rudimentary nephrotome of the fifth segment; T.S. 3.33 mm. embryo (18 somites); × 150. C. Pronephric rudiment in the sixth segment; T.S. 3.45 mm. embryo; × 200. D. Nephrotome and developing tubule; T.S. 4.4 mm. embryo through the sixth segment; × 150. E. Pronephric rudiment in the seventh segment; sectioned 90 μ posterior to Fig. C; ×150. F. Pronephric chambers and developing glomeruli; T.S. 5.24 mm. embryo through the sixth segment; × 150.

ABBREVIATIONS (Figs. 1 and 2):

a, aorta; *ch*, notochord; *ent*, entoderm; *g*, gut; *gl*, glomerulus; *icm*, intermediate cell mass; *lp*, lateral plate; *mb*, mesonephrogenic bridge; *ns*, nephrostome; *nc*, nephrocoele; *n*, nephrotome; *pcs*, postcardinal sinus; *pcv*, postcardinal vein; *pnc*, pronephric chamber; *pnf*, pronephric fold; *s*, somite; *sc*, splanchnocoele; *sd*, segmental duct; *t*, tubule; *vs*, vascular strand.

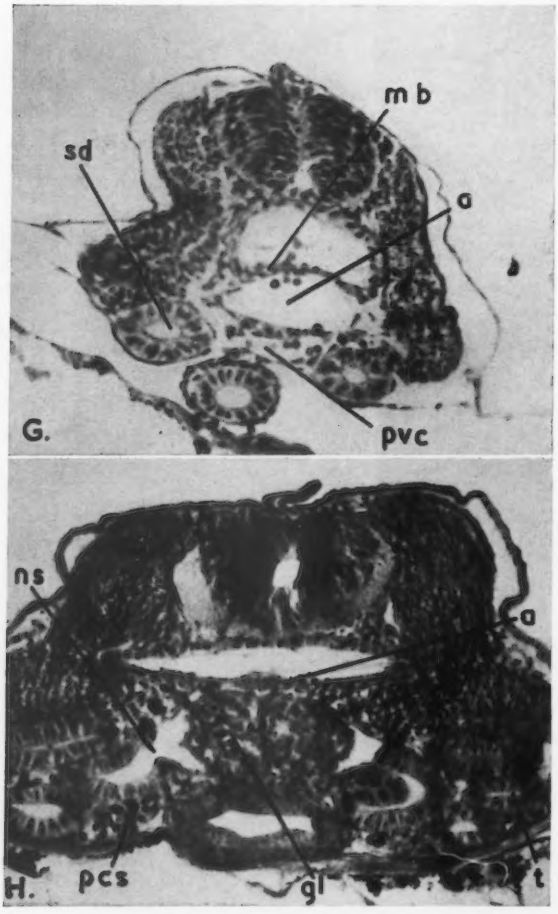

FIG. 2. G. Segmental ducts and blood vessels associated with the posterior trunk region; T.S. 5.24 mm. embryo through the 20th segment; ×200. H. Pronephric chambers and glomerulus in their definitive form; T.S. 9.5 mm. embryo; ×200.

become closely applied to one another (9.5 mm., Fig. 2H). In the vascular strand tissue posterior to the glomerulus appears an accumulation of rapidly dividing basophilic cells from which develops the myeloid tissue of the older embryo. In the 25th to the 27th segments, three pairs of rudimentary corpuscles of Stannius appear as crescent-shaped basophilic outgrowths of the duct epithelium (7.14 mm.).

Further development comprises:

(1) increase in size of pronephric chamber and glomerulus to a maximum length of 400 μ,
(2) increased diameter of chamber and glomerulus to a maximum of 170 μ,
(3) increased vascularity of glomerulus,
(4) elongation of the uriniferous tubule and consequent secondary convolution.

In the 9.5 mm. embryo the spaces between convolutions are filled with myeloid tissue. The mesonephrogenic material appears as a bridge of cells continuous along the trunk and passing from one duct to the other between the cardinal and the aorta. The pronephros attains its peak development at about the time that mesonephric rudiments first appear (16.66 mm. embryo, 50 days). Thereafter, there is little change until involution begins (35 mm., 200 days) considerably later than the appearance of mesonephric glomeruli (25 mm., 105 days).

Involution begins with occlusion of the pronephric arteriole (35 mm.). This is followed by degeneration of the glomerular capillaries and invasion of the glomerulus from either end by fibrous connective tissue. The neck segment of the tubule becomes closed by inward migration of cells from its epithelium. Closure is complete in most specimens of 50 mm.

In a 54 mm. fry, small, well vascularized clumps of suprarenal tissue appear randomly distributed in the pronephric region.

In the largest specimen studied (125 mm., 1 year old) the nephrostomes are closed. The pronephric arteriole is almost entirely occluded. Invasion of its structure by fibrous connective tissue has reduced the length of the glomerulus to 340 μ.

Discussion

Felix (6) claims that the pronephros develops from segmental projections of the lateral plate in *Salmo trutta*. The single pronephric chamber on either side is formed by fusion of three nephrotomes and the tubule by fusion of three metameric tubules. Swaen and Brachet (19) describe a nonsegmental origin from the lateral plate. They deny that the tubule is formed by fusion of three tubule rudiments. Maschkowzeff (11) suggests the delayed separation of intermediate cell mass and lateral plate led to the former author's claim that the pronephric rudiments originate from the lateral plate. Fraser (8) claims segmental nephrotomes do not appear in the development of the teleost pronephros. This study shows the presence of rudimentary segmental nephrotomes in the pronephric segments of *O. gorbuscha*. The pronephric chamber is derived from two fused nephrotomes from which arises a single tubule.

Swaen and Brachet (19) claim that the coelom originally extends into the intermediate mesoderm from the fourth to the sixth segments in *S. trutta*. The opening later becomes constricted to a narrow peritoneal canal which passes from the center of the ventral aspect of the pronephric chamber to the splanchnocoele. Stroer (18) observes a single funnel which opens ventrally to the coelom in *Perca fluviatilis*, but does not discuss its origin. Maschkowzeff (11) claims the fused nephrocoele is at no time continuous with the coelom. In *O. gorbuscha*, the coelom is at first continuous with the fused nephrocoeles of the sixth and seventh segments along their entire length. The opening becomes constricted and closed as medial extension of the coelom rotates the rudiment but at no time do distinct peritoneal funnels form as described by Swaen and Brachet (19).

The first glomerular capillaries in *O. gorbuscha* develop in situ and become secondarily connected to the aorta. The reverse is apparently the case in *S. trutta* where the glomerulus arises as a shoot from the aorta (Swaen and Brachet (19)).

Contrary to the situation in some other vertebrates (O'Connor (12)), the segmental duct in *O. gorbuscha* forms as a fold of the somatic intermediate mesoderm in each trunk segment behind the pronephros. A similar situation is described by Stroer (18) in *Perca*, Maschkowzeff (11) in *Acipenser*, and Swaen and Brachet (19) in *S. trutta*. The latter authors claim that the duct is of both somatic and splanchnic mesoderm and therefore essentially homologous with the pronephric chamber itself. Brachet (4) apparently infers that this mode of duct formation is general in the teleosts, and peculiar to that group.

Summary

In *O. gorbuscha* the rudimentary nephrotomes of the second to the fifth segments become incorporated into the coelomic wall. The intermediate mesoderm of the pronephric region is segmented but opposite the sixth and seventh somites its segmental character is obscured. After the nephrotome in this region becomes separated from the lateral plate the enclosed cavity dilates to form a single pronephric chamber on either side. The medial walls of the two chambers become closely approximated in the mid-line. A single elongate glomerulus formed by fusion of the glomeruli of either side invaginates the medial walls of the chambers. A solid outgrowth of the wall of the chamber becomes tubular and enters the segmental duct which separated from the intermediate mesoderm in the more posterior segments. In later stages the tubule epithelium is distinguishable from that of the duct by a distinct brush border. The pronephros persists for a considerable time after the appearance of functional mesonephric glomeruli, though signs of degeneracy are apparent.

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THE SALIVARY GLAND CHROMOSOMES OF TWO SIBLING SPECIES OF BLACK FLIES INCLUDED IN EUSIMULIUM AUREUM FRIES[1]

ROBERT W. DUNBAR

Abstract

Two closely related dichromosomic sibling species of *Eusimulium "aureum"* were segregated cytologically in populations sampled from the Toronto area. The known range of these two so far included southern Ontario and Quebec, and New York State. Both siblings have extremely similar salivary gland chromosomes but they differ by four homozygously rearranged regions, all in the first chromosome, and in the degree of chromosomal polymorphism. Although sympatric over a wide range, the two siblings do not interbreed as evidenced by the failure to find critical heterozygous loops for the interspecific inversions, and the absence of common intraspecific inversions. Therefore the siblings represent two biologically distinct species.

Introduction

This study is a result of the cytological survey of the Simuliidae of Eastern Canada begun in Toronto in 1951. In the intervening years considerable cytological information accumulated which indicated that *Eusimulium aureum* Fries is a sibling species complex rather than a single discrete species. The discovery of sibling species is not new (19), many instances having been reported in cytotaxonomic studies of *Drosophila* (4, 21, 38, 48). Similarly the preliminary cytological studies in black flies have indicated a number of situations in which a morphologically discrete species consists of two or more distinct cytological forms which do not appear capable of interbreeding. One such case already reported refers to two sympatric sibling species, *Prosimulium "hirtipes", 1* and *2* (27), which differ cytologically in two homozygous inversions, the details of the sex determining mechanism, and characteristic sets of floating inversions. Recently morphological distinctions in the adults have been found (42).

Eusimulium "aureum" siblings are distinct from all other black flies so far studied in that they have only two chromosomes. Furthermore two siblings were distinguished in the first eight slides in which this dichromosomic condition was recognized. They differed in several homozygously inverted regions and the frequent occurrence of heterozygous inversions in one sibling only. The existence of these two sibling species was subsequently fully confirmed with larger population samples for the Toronto area.

In the Montreal area the same two forms found in the Toronto area are present. *Eusimulium "aureum"* from Churchill, Manitoba, appears to separate cytologically into two additional siblings species, C and D. In Berkeley,

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California, there is found a fifth sibling. Population analyses for these localities are incomplete, and are referred to only in essential context in the discussion. The bulk of this paper deals with the analysis of larvae of siblings A and B in the Toronto area.

Materials

Eusimulium aureum was described by Fries in 1824 from material collected on an expedition to Lapland in 1821 (13, 14). Since then it has been reported from widely scattered areas throughout the holarctic zone. Its range is from the treeline (16, 37, 39, 44) to Guatamala (7, 45) in North America; throughout Europe (20, 23, 25); in the Atlas Mountains of North Africa (15); throughout Russia (10, 31); and in the Siwalik Hills in India (26). Further lists of reports and descriptions are given by Stone and Jamnback (40), and Smart (36).

Larvae of *E. "aureum"* were first collected in connection with this cytological survey in Churchill, Manitoba, in the summer of 1951, but were not identified at the time, nor were good preparations obtained. In the spring of 1952 in a branch of the Dixie Creek just north of Cooksville, Ontario, larvae were found of a species that had only two chromosomes (Table I). They were incorrectly identified as *Eusimulium euryadminiculum* Davies and reported as such (29). This was corrected in the fall of 1953 when associated pupal material was identified by Shewell (32) as *E. aureum* Fries (33, 34).

The larvae of *E. "aureum"* siblings are typically found in small, clear, relatively slow lowland streams, usually with a clean bottom interspersed with vegetation, from early May into October (Table I). The larvae attach to submerged vegetation or stones, etc. They are never very plentiful but with a little patience reasonable sized samples may be obtained. This species did not appear until after the streams had warmed up in the spring (40, 43). In the Toronto area all *"aureum"* streams but the Dixie Creek were permanent. Twinn (43) found *"aureum"* larvae in similar but semipermanent or temporary small streams.

Eusimulium "aureum" is not restricted entirely to warm, small streams. The material from Montreal (Table I) was collected in shallow rapids of the St. Lawrence River (50).

The author also collected larvae of sibling C from a cold tundra stream that appeared to receive its water from two small tundra pools draining over melting permafrost.

The seven Toronto area localities collected from (Table I) were (1) a branch of the *Dixie Creek** at a point 0.5 miles east of Highway 10 on "Back Line Road", 1.3 miles north of Cooksville, (2) Dentonia Park, Toronto, (3) a very small permanent stream 2 miles due west of *Terra Cotta*, (4) a small stream 0.5 miles due north of Terra Cotta, and three sites on Highway 400, (5) 0.4 miles south of the Alliston cutoff, (6) 4 miles south of the Alliston cutoff on the east side of *Highway 400*, and (7) the top of south slope leading to the *Holland Marsh*.

*Place names in italics refer to principal collecting sites.

The best salivary gland chromosomes come from fully matured larvae, those showing pupal respiratory histoblasts, but younger larvae also yield workable preparations (3, 22).

Using the cytological markers described later for the long chromosome, only one slide could not be definitely identified as to type. The 435 preparations of type B larvae could be completely categorized. Of the 209 type A slides, 191 were sufficiently clear to allow complete classification of inversions in all arms.

TABLE I

COLLECTIONS IN THE TORONTO AND MONTREAL AREAS SHOWING SAMPLE SIZE, LOCATION, AND DATE

Location	Collection date	Frequency of sibling A	Frequency of sibling B
Dixie Creek	May 7, 1952	2	6
	May 4–20, 1954	6	27
		(8)	(33)*
Dentonia Park	June 23, 1953	(8)	(3)
Terra Cotta	Oct. 1, 1953	4	1
	May 24, 1956	6	1
	June 1, 1956	29	3
	Sept. 30, 1956	23	46
		(62)	(51)
N. of Terra Cotta	June 1, 1956		(4)
Alliston cutoff	May 29, 1956		(1)
Highway 400	May 29, 1956	1	14
	June 6, 1956	43	10
	June 30, 1956		45
	July 7, 1956	5	98
		(49)	(167)
Holland Marsh	May 27, 1956		1
	May 29, 1956	6	34
	June 6, 1956	1	8
	June 30, 1956	7	25
	July 7, 1956	18	5
	Aug. 27, 1956		3
	Sept. 18, 1956	37	68
	Oct. 25, 1956	8	17
	Oct. 30, 1956	5	15
		(82)	(176)
		209	435
		644	
Montreal, Que.	June 23, 24, 1953	1	7
	June 8, 1954	1	
Prefontaine, Que.	July 19, 1953	2	
	Totals	213	442
		655	

*Parentheses denote location totals.

Pupae were collected at some localities at the same time as the larvae, and adults reared from them, but no attempt was made to rear adults from larvae of either sibling. This material is on hand but has not been submitted to a specialist for examination.

Morphological Distinction of the Larvae

The larvae of *E. "aureum"* have been described or illustrated by a number of authors (11, 15, 24, 35, 37, 40): *Eusimulium bracteatum* (Coquillet) (17, 18, 41). Identification of living larvae of *"aureum"* was principally on their reddish color, the head pattern, and gular (throat) cleft (37, 40). Slight differences in these last two characters can be used to sort out siblings A and B.

Larvae of type A tend to have more pigment around the head pattern spots than larvae of B in the same stream. The over-all amount of pigment varied from stream to stream so this was never used, except once as a way of concentrating (Holland Marsh, Sept. 18, 1956). Cytological examination showed a 48% error for A but only 2% for type B.

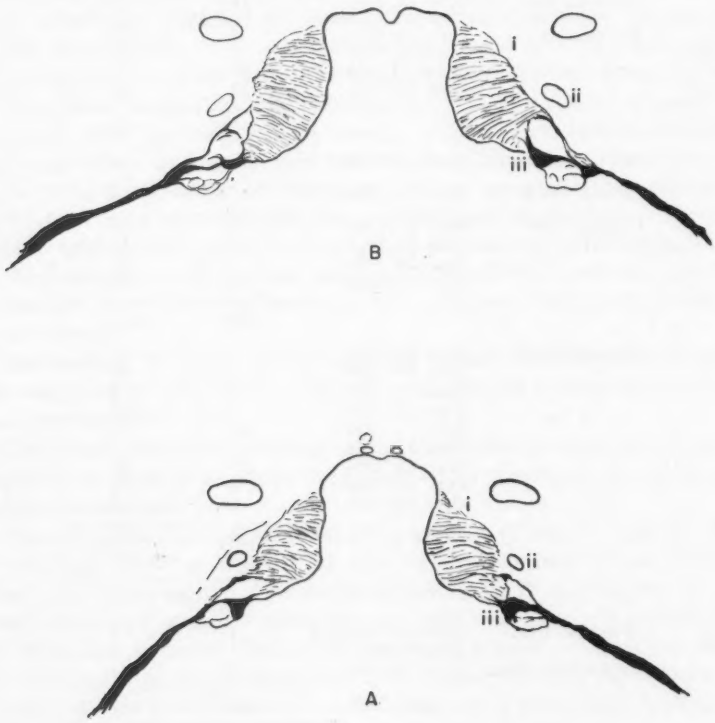


FIG. 1. Diagrams of the gular cleft of *E. "aureum"* siblings A and B. (*i*) Lateral chitin deposit, (*ii*) lateral spot, and (*iii*) "papillae".

In over-all appearance the gular cleft of sibling A is rounded, of B, somewhat rectangular (Fig. 1). The flatter appearing top of the gular cleft in B does not always have the papilla-like central projection illustrated whereas the upcurved top in A never has more than an indefinite irregularity.

Three samples were provisionally typed after staining, using differences in the gular cleft. Subsequent cytological examination showed error from 6% to 10% (Table II).

TABLE II

VALIDATION OF GULAR CLEFT IDENTIFICATION OF SIBLINGS
(*F'* number of larvae correctly identified by gular cleft in sample of size *F''**)

	Sibling	*F'*	*F''*	% error
Highway 400	A	21	23	8.3
June 6, 1956	B	1	1	
Holland Marsh	A	31	34	9.5
Sept. 18, 1956	B	57	64	
Terra Cotta	A	21	23	5.8
Sept. 30, 1956	B	44	46	

*Not all larvae in the first two collections were preclassified morphologically.

Mechanical damage and stain made identification by gular cleft more difficult than it would have been had the larvae been alive or preserved intact. The author feels the gular cleft characteristics will provide the basis for a proper morphological separation of siblings A and B.

In their description of *E.* "*aureum*" larvae, Stone and Jamnback (40) state of the gular cleft "apex flat *or* slightly upcurved medially". This corresponds to the illustrated difference for the apex (Fig. 1) and suggests that both siblings A and B are also found in New York State. Other minute differences about the gular cleft were noticed: (*i*) The size of the lateral spot, (*ii*) the pattern of deposition in the chitin laterally, and (*iii*) the shape of the heavily chitinized paired "papillae" and its attendant structures (Fig. 1). These differences were found to be constant in five larvae of sibling A and in seven of B.

Methods

The method of preparation of slides was initially as used by Rothfels and Dunbar (29) with certain later changes and additions. Two per cent orcein in 60% acetic acid was also used for both chromosomes and nucleolus. Larvae were fixed in Carnoy and hydrolyzed in cold 1 *N* HCl for 6 minutes. Material fixed in Carnoy can be stored several days especially if it is kept cold. This means material fixed in one location may be air-mailed to another for preparation. Feulgen stained larvae following the SO_2 water rinse were then washed in several changes of tap water and left a few minutes. This tended to intensify the stain and toughen the chromosomes so they would not break so

readily in squash mounting. Material could be left about a day in tap water if kept cool. The acetic acid temporary mounts were made permanent by the dry-ice method of Conger and Fairchild (6).

Temporary mounts of sibling B were rejected as soon as it was established that there was nothing unusual about the chromosomes—only a few good ones were kept. All those of type A were made permanent for more leisurely scrutiny. The cadavers of all larvae were kept by gluing them in order to slides.

Photography and measurement of salivary gland chromosomes were as by Rothfels and Dunbar (29), except that a "map measurer" was used to obtain lengths of camera lucida tracings.

Larvae collected in the summer stained weakly with both Feulgen and orcein procedures. Feulgen stained material was therefore often restained with orcein in an attempt to intensify the over-all stain. This difficulty was not present in larvae collected in the cooler spring or autumn (30).

Results

Mitotic Complement

An examination of mitotic metaphases from neuroblasts or gonial cells from both siblings showed two pairs of somatically paired metacentric chromosomes (Fig. 3). Camera lucida tracings were measured to find the length in microns of the mitotic chromosomes. The noticeably longer pair (No. I) is 7 μ, the shorter pair (No. II) 4 μ. The arm ratios about the primary constriction are approximately 3:4 in the longer chromosome and 1:1 in the shorter.

General Features of the Salivary Gland Chromosomes

Because of the remarkable similarity between siblings A and B, the general description of the giant chromosomes is applicable to both. As could be expected from the mitotic complement, there are only two pairs of salivary gland chromosomes. The two constituents are very tightly wound around each other so the pair appears as one unit (Fig. 2). Only occasionally is there a break in the pairing (Fig. 11: sections 22, 23). The ends of the chromosomes in this species complex tend to random ectopic pairing, to give odd configurations such as two chromosome chains (Fig. 2), figures-of-eight, separate loops, etc.

The expanded region of the long chromosome (Figs. 5, 10–16) typically appears to be two to four times the diameter of the rest of the chromosome. That part of it from 24B to 27B has a loose, large-chromomeric or tufted appearance somewhat as in *Simulium vittatum* Zett. (29). Near the limits of this region two conspicuous deeply staining bands are found. The expanded

FIG. 2. Complete standard salivary gland complement of sibling B. The arrow points to the point of fusion of the IL and IIS ends. × 1500

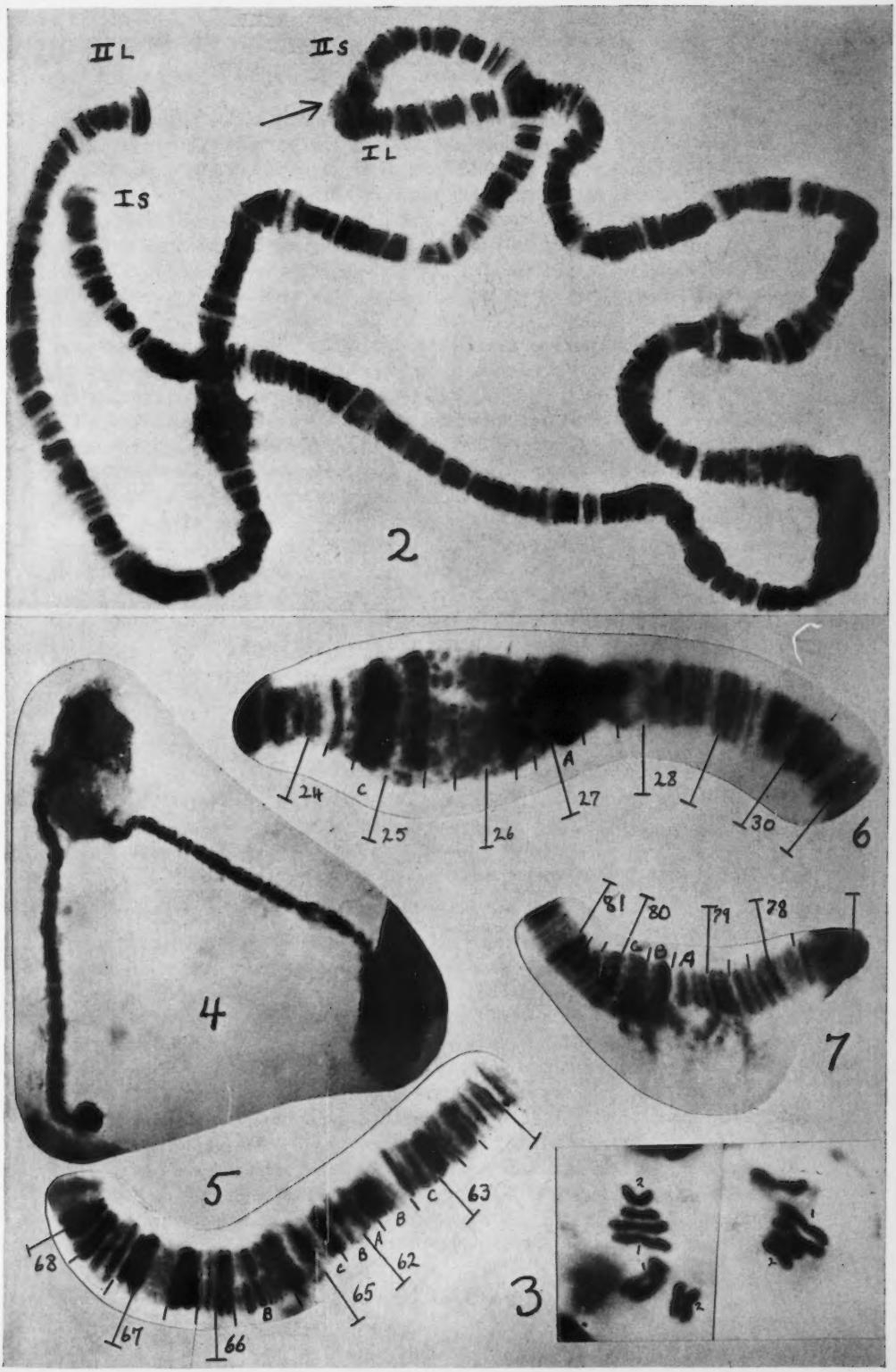
FIG. 3. Somatic complement from gonial cells. Numbers identify chromosomes and centromeres. × 2400

FIG. 4. Chromosome II, nucleolus. × 550

FIG. 5. Chromosome II, sibling A, discrepancy in segment 66B. × 2200

FIG. 6. Chromosome I, expanded region. × 2300

FIG. 7. Chromosome II, expanded region. × 2300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region tapers off to the diameter of the long arm gradually through a series of indistinct bands (Figs. 14–16) whereas the transition into the short arm is abrupt (Figs. 10–12). The single Ring of Balbiani (29) is found in the long arm of this chromosome. It's position (44B) differs in the two siblings (Figs. 14–16) and will be discussed later. The expanded region in the shorter chromosome is located almost in the center of the chromosome (Fig. 8). It is similar in size and structure to the expanded region of *S. vittatum* (29). In addition to a heavy dark band, the expanded region contains on the short arm side, the nucleolus (Figs. 7, 17–25), which is occasionally expressed only in one constituent (Fig. 4). The nucleolus sometimes expands so as to obliterate the internal expression of the expanded region making it difficult to tell the relation of the dark band to the nucleolus (Figs. 22, 23).

Both salivary gland chromosomes are subdivided into arms by the expanded regions (Figs. 4, 5). To find the ratios of these arms nuclei from both siblings were measured. The per cent total complement length (% TCL) (27, 29) of the various segments was calculated (Table III) and presented graphically (Fig. 8). Since the resulting arm ratios approximate those of the mitotic chromosomes, the expanded regions of the salivary gland chromosomes are homologized with the mitotic centromere region.

TABLE III

MEAN POLYTENE COMPLEMENT OF SIBLINGS A AND B IN % TCL

A: 10 nuclei, two each from five larvae representing two localities
B: 10 nuclei, two each from five larvae representing four localities

	IS*	IL	IIS	C–N†	N–IIL
Sibling A	26.5	35.8	17.7	0.5	19.2
Sibling B	24.3	35.8	19.0	0.4	20.6
Mean	25.4	35.8	18.4	0.5	19.9

*Long chromosome divided into arms midway between the two centromeres.
†N, center of nucleolar gap; C, centromere.

There is no direct evidence to indicate the centromeres in the salivary gland chromosomes of *E. "aureum"*, but there are heavy dark bands in the expanded regions which are homologized with the centromeres of *S. vittatum*. The position of the centromeres has been confirmed in *vittatum* by frequent ectopic pairing of the heavy bands in the expanded region (29) and in some *Prosimulium* and *Eusimulium* by ectopic pairing or chromocentric fusion (28). But in *E. "aureum"* there are two heavy bands in the "tufted" part of the first chromosomes' expanded region (Figs. 6, 10–16: 24C, 27A) either of which could be considered the centromere. Since the "tufted" part of the expanded region is at least twice the length of any comparable expanded region in other black flies, this suggests that both bands are in fact centromeres. If this is so, the first chromosome of *E. "aureum"* is dicentric. The second chromosome has the usual single heavy band in a "normal" appearing expanded region.

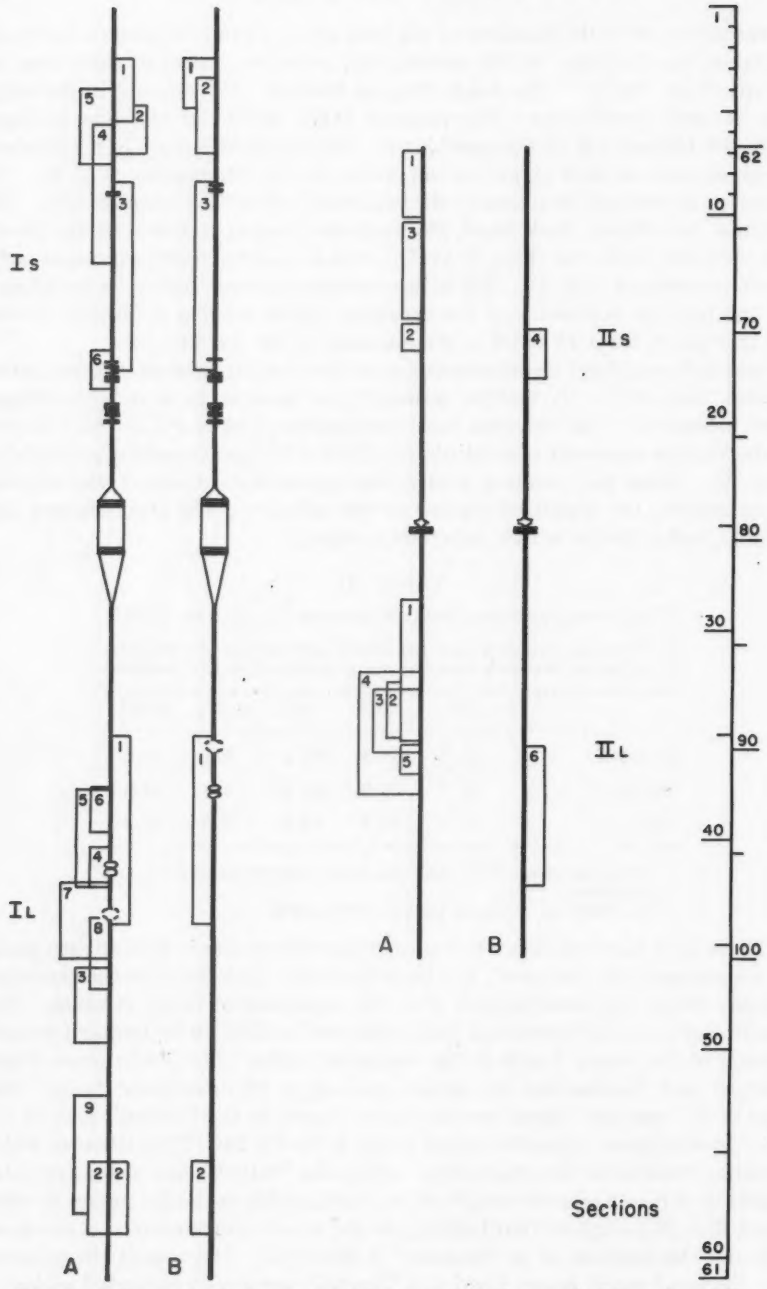


FIG. 8. Salivary gland chromosome idiograms of *E. "aureum"* siblings A and B.

The Idiogram

The idiogram (Fig. 8) for arm length, position of the nucleolus, and expanded regions is based on the measurements of Table III. To find other points, the 100 sections of the chromosome were assumed equal. The various cytological landmarks and inversion limits were then plotted according to their sectional positions. Consequently there are slight errors of no more than 0.5% between the measured % TCL distances and the idiogram representation.

Plate Nomenclature

Since it proved possible to carry out a band for band comparison of the two siblings throughout the chromosomes, it was decided to use the same section limits to simplify nomenclature. The sibling with the larger number of floating inversions, A, was chosen as the standard and the other sibling, B, compared to it. The standard sequence is that arrangement in sibling A which has the most in common with the arrangement found in type B. The remaining differences constitute the interspecific inversions. Because of the large number of floating inversions found in type A a complete homozygous standard complement was not found. One slide was nearly so except that the inversion IIS-1 was involved heterozygously with standard.

The division of the salivary gland chromosomes for mapping purposes follows the pattern used by Rothfels (27). The chromosomes are divided into short and long arms by the centromere region, the four arms being IS, IL, IIS, IIL. The whole complement is then divided into 100 approximately equal primary sections beginning in IS, and continuing through IL. Each section is subdivided into three subsections—A, B, C.

On the plates, to eliminate congestion, every other section is numbered and very few of the segments are labelled. Missing numbers or letters may be identified by interpolation between labelled segments on either side.

For a given arm all inversions are numbered consecutively from one whether they are interspecific or floating in sibling A or B. The intraspecific inversions are represented in print by Roman type (IL-6, IIS-3), interspecific by italics (*IS-3*, *IL-1*). On the maps and idiograms (Figs. 8, 10–25) the inversions are represented by brackets (1). Those away from the opposed sibling and marked in one sibling only represent intraspecific inversions; those towards the opposed sibling and marked on both represent interspecific inversions (Fig. 8). Where the bracket originates at the chromosome it represents a simple inversion with respect to standard (for either A or B) even though it may overlap other inversions on the map. A bracket stopping on another bracket represents a break occurring in a segment already displaced in standard and yielding overlapping (IL-2.9) or included (IIL-1.2) sequences. Coincident breakpoints are shown by a common line (IL-4, IL-5).

Descriptions of the Arms

As the banding patterns are so similar the four arms of both siblings are examined in pairs, i.e. IS of A with IS of B, etc. The description will cover (1)

means of distinguishing the arm in question, (2) qualitative characteristics that distinguish A from B, (3) detailed analysis of the interspecific inversions between the siblings, and (4) intraspecific inversions.

First Chromosome, Short Arm, IS

The limit of this arm is taken at a point midway between the two dark bands of the centromere region. The arm is identified by the three heavy double bands in section 20 (Figs. 8, 10–12) in all siblings from any area so far examined. From the expanded region to 19B the sequence for all siblings is the same. At this point siblings A and B differ in respect to the interspecific inversion *IS-3*. This inversion has two heavy bands (section 18A1–B3) at one end and at the other a characteristic chromosome segment (section 9B–C) starting with a "gap", or clear region (Figs. 8, 10–12). When the segment 18C-B4 is preceded by the two heavy bands of *IS-3* (18A1–B3) then the sibling

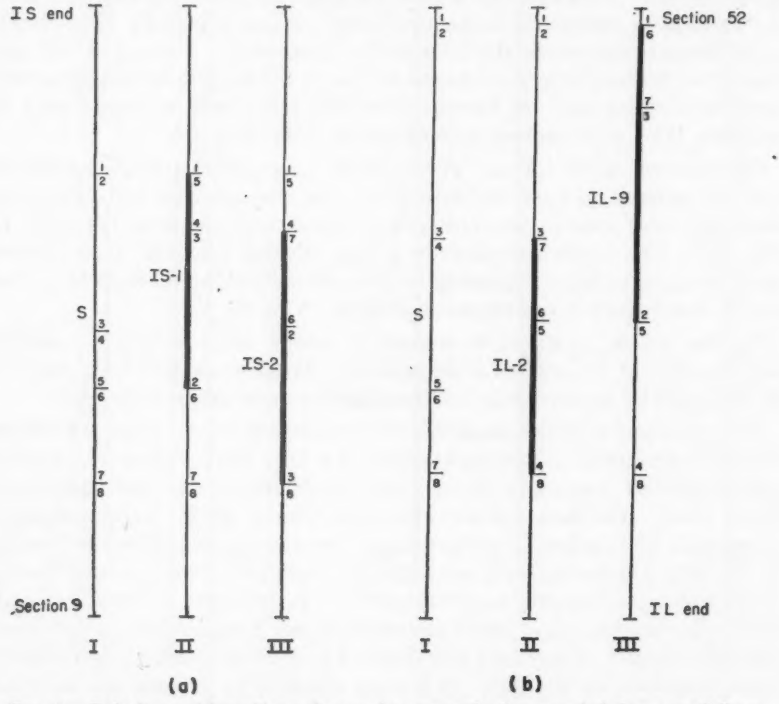


FIG. 9. Relative position of inversions and segments in the two triads between siblings A and B in the long chromosome.
(*a*) S⇋IS-1⇋IS-2
I. Standard in A.
II. Hypothetical between A and B, IS-1 (standard in C and D).
III. Standard in B, IS-1.2.
(*b*) S⇋IL-2⇋IL-9
I. Standard in A.
II. Standard in B, IL-2 (hypothetical in A).
III. Rare arrangement in A, IL-2.9.

is A; when 18C–B4 is preceded by the gap then it is B. This distinction was one of two (the other, the position of the Ring of Balbiani) used with confidence for very poor preparations. Since no floating inversions were found in B in this arm the presence of a heterozygous loop was also used to help identify sibling A.

TABLE IV

DESCRIPTIONS OF THE INVERSIONS FOUND IN THE SALIVARY GLAND CHROMOSOMES OF SIBLINGS A AND B

(*Interspecific* and intraspecific inversions are described in terms of the standard A sequence. Arrangements are described by giving the end bands of the rearranged segment or segments along with the end bands of the standard sequences between which the rearrangement occurs, the segments being separated by periods. *F'*, frequency of homozygotes; *F''*, frequency of heterozygotes*; % of constituents)

Arrangement	Description of arrangement	*F'*	*F''*	(%)
	First chromosome, short arm (IS)			
Standard A	1A1–25C			
Standard B	1A1–3B1.6B2–5C1.7C3–6B3.3B2–5B4. 8A1–9A4.18B3–9B1.18B4–25C			
IS–1.2	3B1.6B2–5C1.7C3–6B3.3B2–5B4.8A1			
IS–3	9A4.18B3–9B1.18B4			
IS–4	6B2.13B1–6B3.13B2	57	88	52.8§
IS–5	4C1.8B1–4C2.8B2		4	1.0
IS–6	17B.19A5–17C1.19B			†
	First chromosome, long arm (IL)			
Standard A	26A–61C			
Standard B	26A–35C.44C5–36A1.45A1–56A4. 59C2–56B1.59C3–61C			
IL–1	35C.44C5–36A1.45A1			
IL–2	56A4.59C2–56B1.59C3			
IL–2.9	52C.59A1–59C2.56A4–53A1. 58C5–56B1.59C3		2	0.5
IL–3	47A.50B3–47B1.50C1	11	101	32.2
IL–4	41A.43A2–41B1.43A3	1	28	7.9
IL–5	38B.43A2–38C1.43A3		8	2.1
IL–6	38B1.40B3–38B2.40C1		3	0.8
IL–7	42C.46C4–43A1.47A1		2	0.5
IL–8	44B.48A2–44C1.48A3		1	0.3
	Second chromosome, short arm (IIS)			
Standard A and B	62A1–80A			
IIS–1	62A2.65B2–62A3.65B3	157	33	90.8
IIS–2	65B5.70A4–65C1.70B1		23	6.5
IIS–3	70B2.71C2–70B3.71C3		1	0.3
IIS–4 (B)	70C4.73A2–70C5.73A3		5	0.6
	Second chromosome, long arm (IIL)			
Standard A and B	80B1–100C			
IIL–1	83C4.90B5–83C5.90B6	10	75	24.8‡
IIL–1.2	83C4.90B5–90B4.87C2–90B3. 87C1–83C5.90B6		10	2.6
IIL–3	88A2.91A2–88A3.91A3		13	3.4
IIL–4	87B1.93A2–87B2.93A3		5	1.3
IIL–5	90C1.92A1–90C2.92A2		1	0.3
IIL–6 (B)	90C1.97B1–90C2.97B2		2	0.3

*191 sibling A, 435 sibling B.
†In two larvae from Prefontaine, Que.
‡Including IIL–1.2.
§All arrangements, other than the inversion being examined, are considered as standard.

Sibling A differs from B in two regions in this arm. One of these is the simple inversion *IS-3*, involving nearly 40% of the arm, and providing the distinguishing landmarks already discussed. In a second subterminal region A differs from B by two overlapping inversion steps, *IS-1* and *IS-2* (Figs. 8, 10–12; Table IV) forming a triad (46, 47) where the intermediary is not known from southern Ontario or Quebec (Fig. 9*a*). On the maps and idiograms *IS-1.2* is shown by two overlapping brackets. The intermediary form, *IS-1*, does however occur at Churchill, Manitoba, in association with the A arrangement of *IS-3* in the short arm and the B arrangement in the long arm in sibling C and a new but undetermined arrangement in the long arm in larvae of sibling D.

Only one of the floating inversions in sibling A was common, IS-4, appearing in 53% of the constituents (Table IV). IS-5 was rare being found four times heterozygously. It is independent of IS-4 but overlaps it on the map forming as it were another triad IS-4 ⇆ S ⇆ IS-5.* IS-5 was found once in an overlapping configuration with IS-4. IS-6 was found in two larvae from Prefontaine, Quebec, but was included on the map because of the relative proximity to the Toronto area and its possible occurrence in southern Ontario.

First Chromosome, Long Arm, IL

Apart from length, two points serve to distinguish this arm from the shorter. First, from the second dark band of the centromere region to approximately section 29 the expanded region appears somewhat cottony and gradually tapers to the normal chromosome diameter (Figs. 8, 14–16). The other distinguishing feature is the Ring of Balbiani (R.o.B.) (section 44B).

The R.o.B. also serves to distinguish sibling A from B since it is involved in the interspecific inversions *IL-1* (Figs. 8, 14–16; Table IV). In consequence in A the R.o.B. is found approximately in the center of the arm whereas in B it is located about one-quarter the length of the arm from the centromere. In addition there is the "double bubble", a heterochromatic region (section 42), with a prominent constriction (Figs. 14, 15, 16: between 42A and 42B) dividing the region into two lobed or "bubble-like" halves. This conspicuous marker region is proximal to R.o.B. in sibling A and distal to it in B.

Siblings A and B again differ in two regions in this arm. In the central region they differ by the simple inversion *IL-1*, which has the R.o.B. and "double bubble". Secondly, in the subterminal portion (section 52B1–59C2) three sequences are known that differ by simple inversion steps to form an overlapping triad (Fig. 9*b*). If the common sequence of A is chosen as standard, the central sequence *IL-2* is confined to sibling B as its sole arrangement

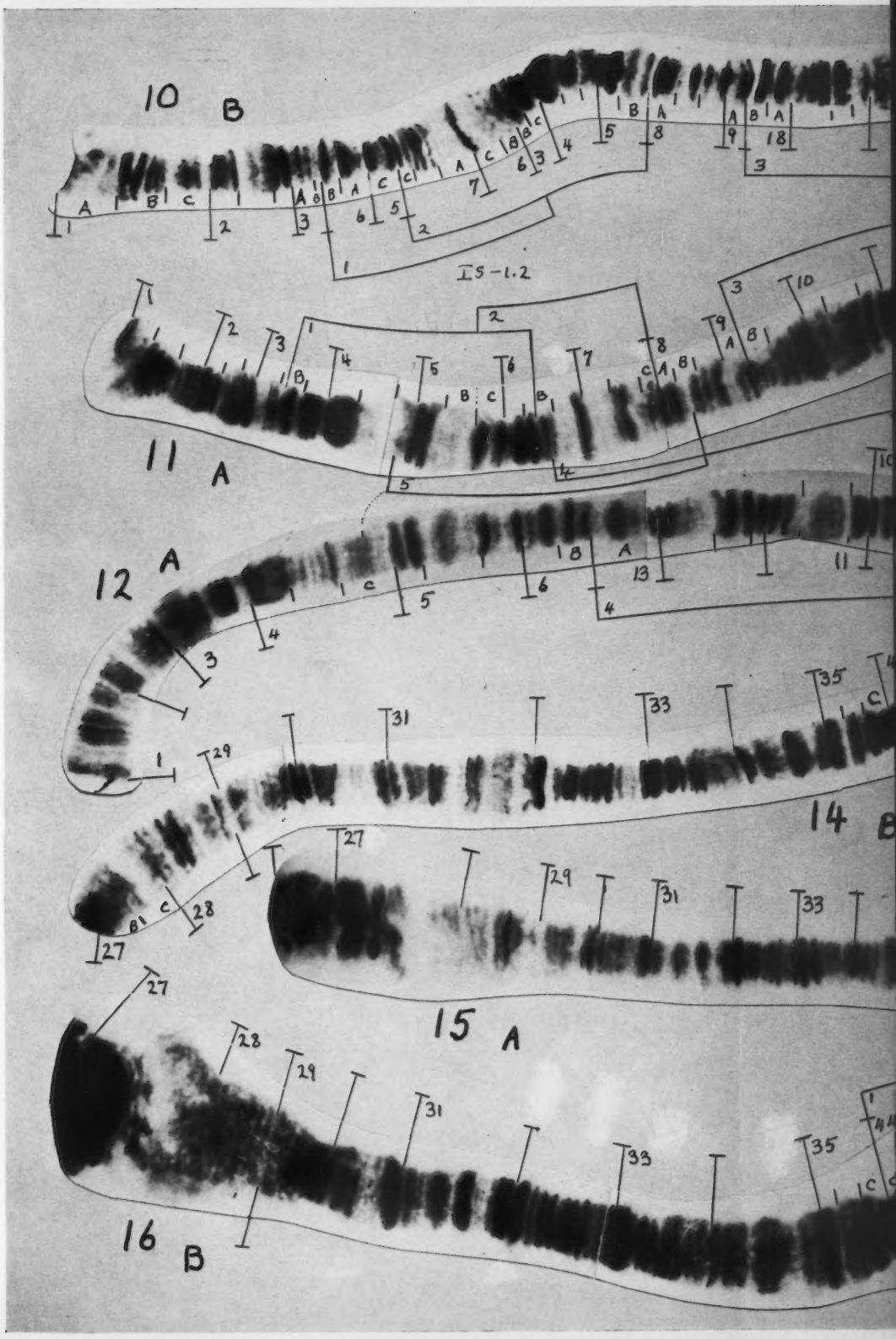
* S, standard arrangement in sibling A.

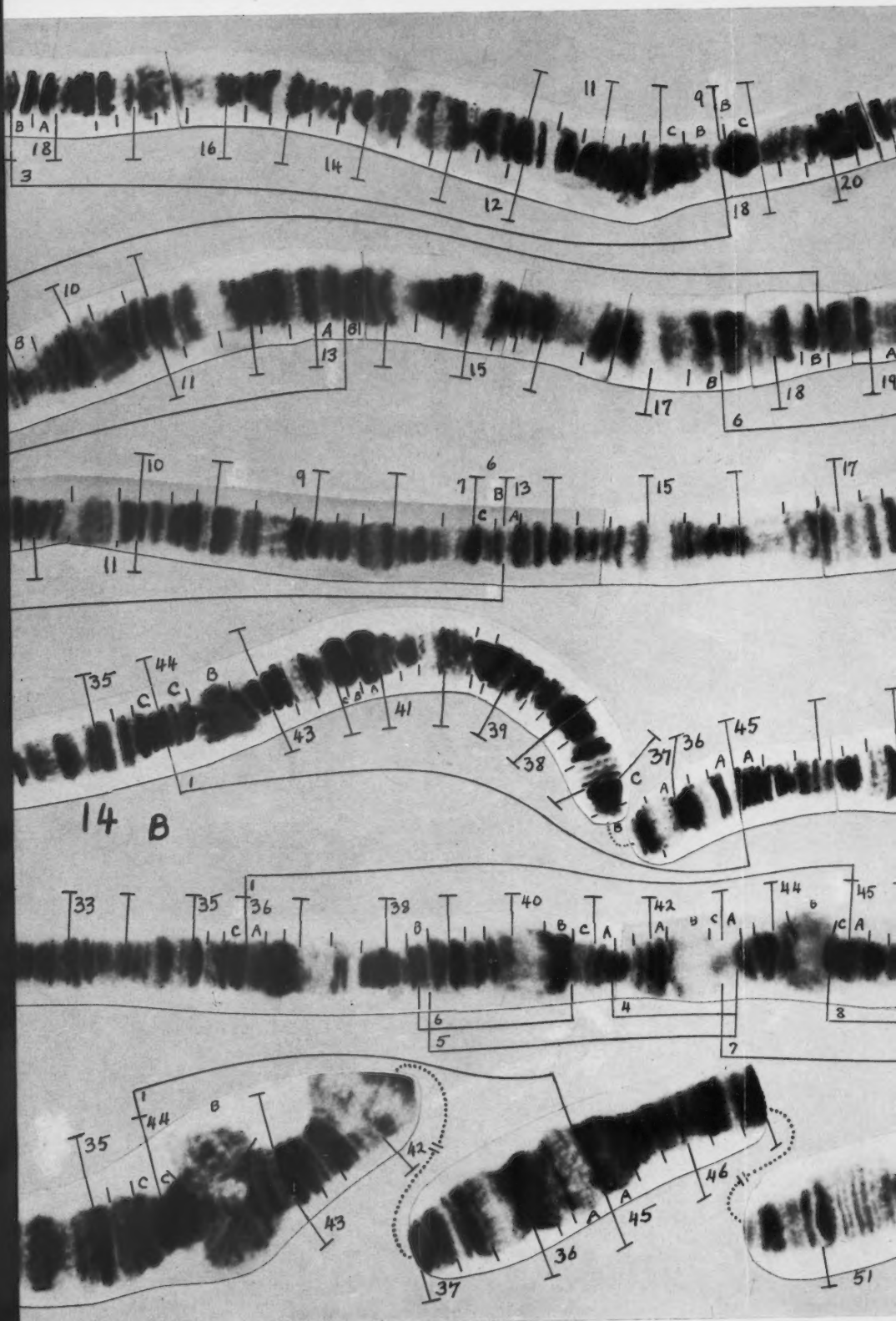
FIGS. 10–13. Chromosome I, short arm, IS.

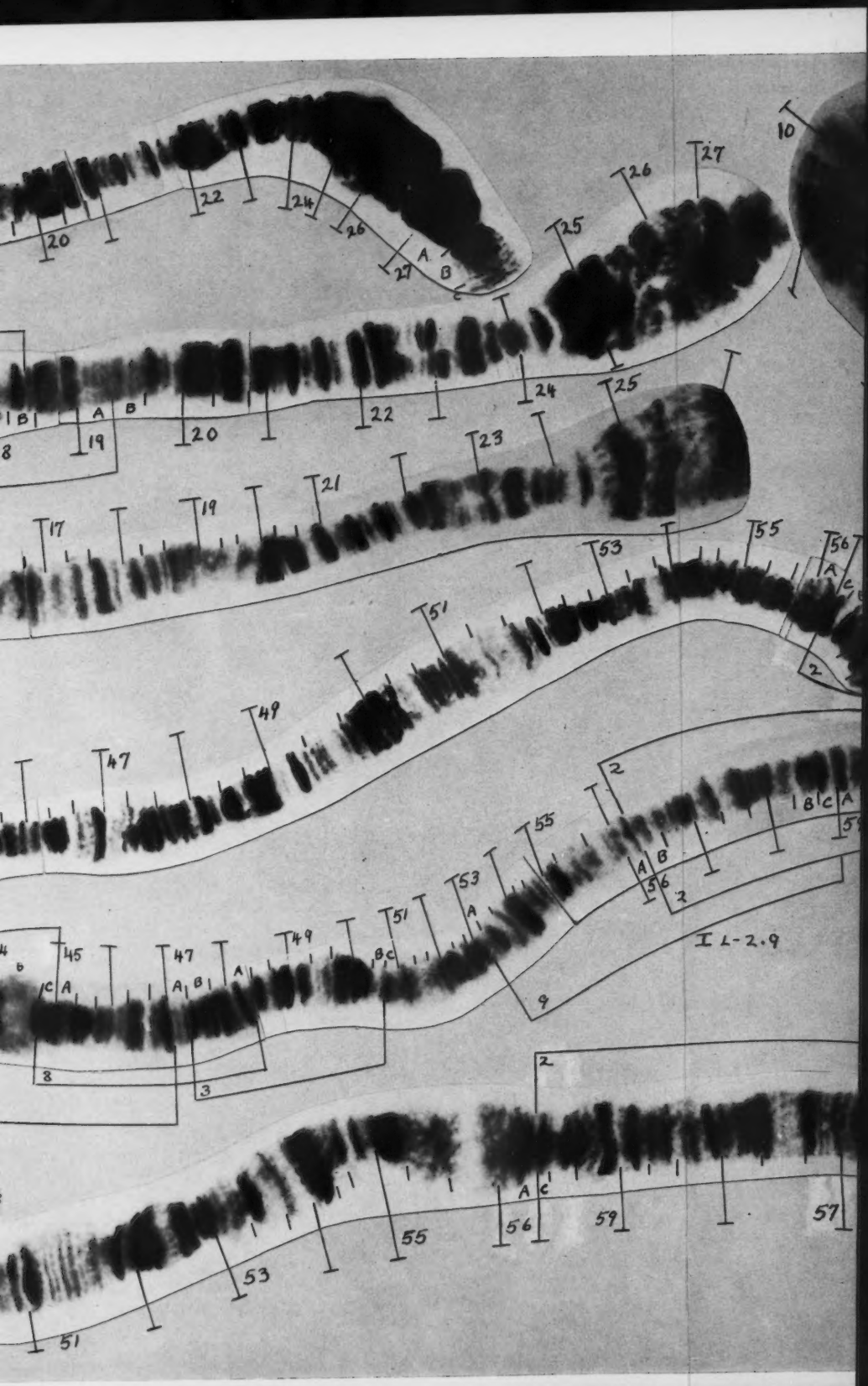
FIG. 10. Sibling B, standard band sequence. FIG. 11. Sibling A, standard band sequence. FIG. 12. Sibling A, heterozygous for inversion IS–4. FIG. 13. Sibling A, homozygous for inversion IS–4.

FIGS. 14–16. Chromosome I, long arm, IL.

FIGS. 14, 16. Sibling B, standard band sequence. FIG. 15. Sibling A, standard band sequence.


10 B
IS-1.2
11 A
12 A
14 B
15 A
16 B


14 B


10
20
22
24
26
27
A
B
C
25
26
27
24
25
22
20
19
23
21
17
19
53
55
56
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49
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2
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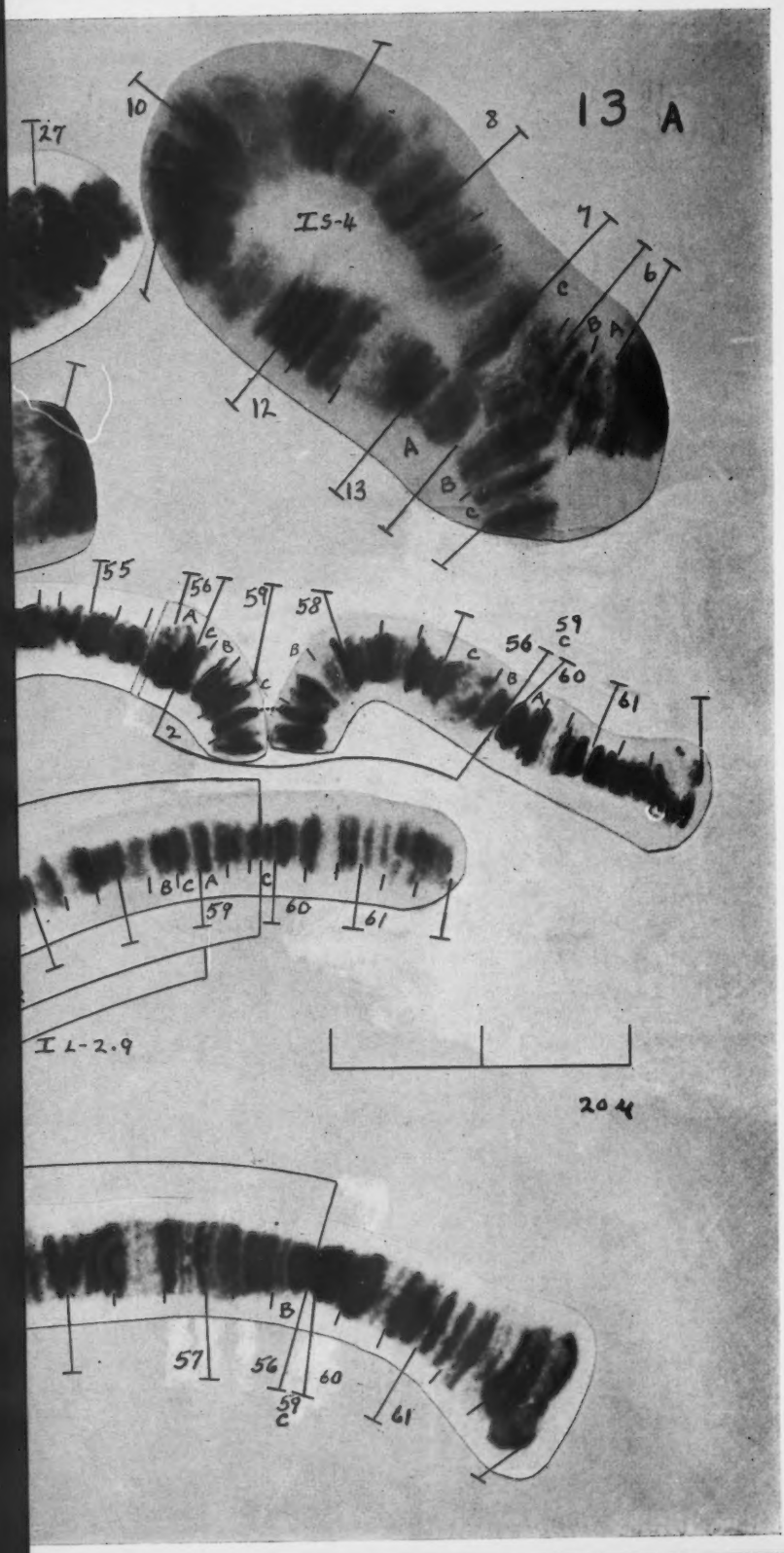
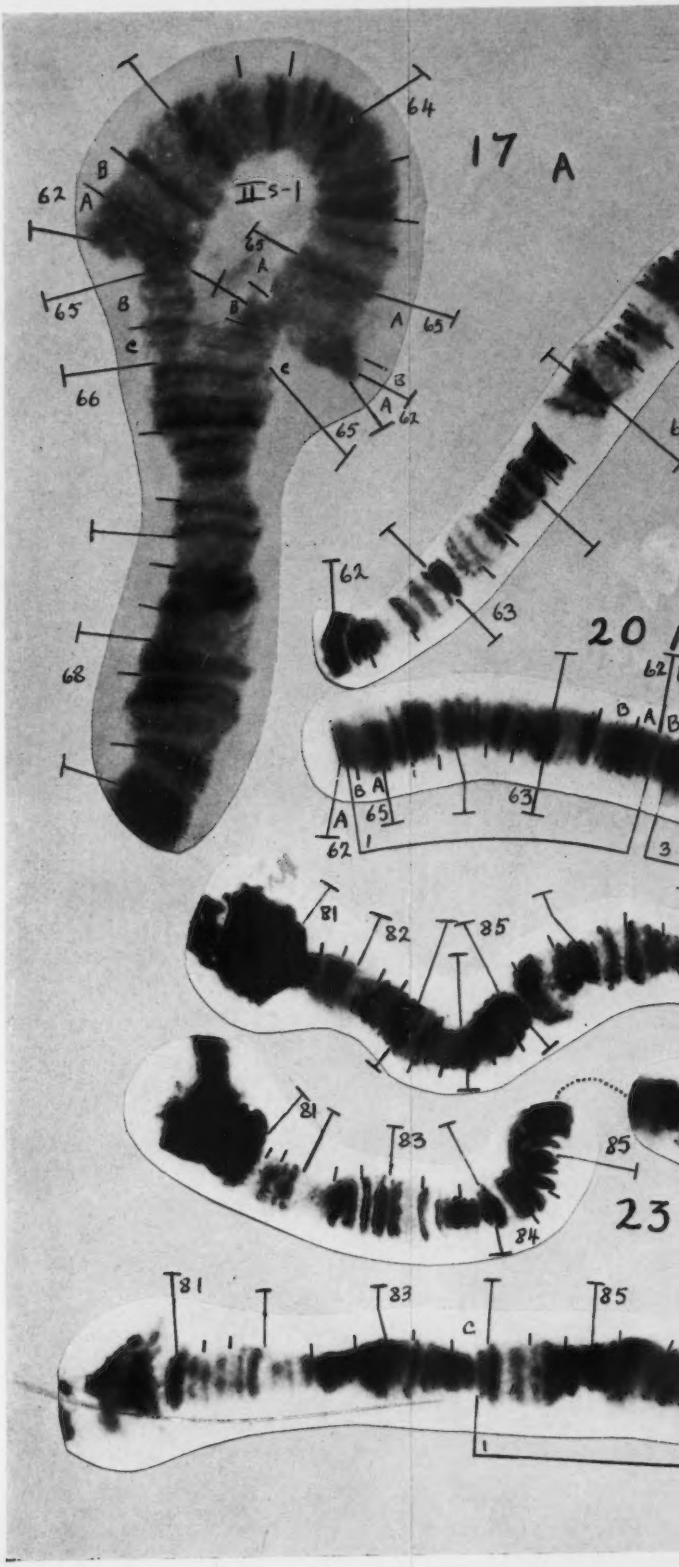
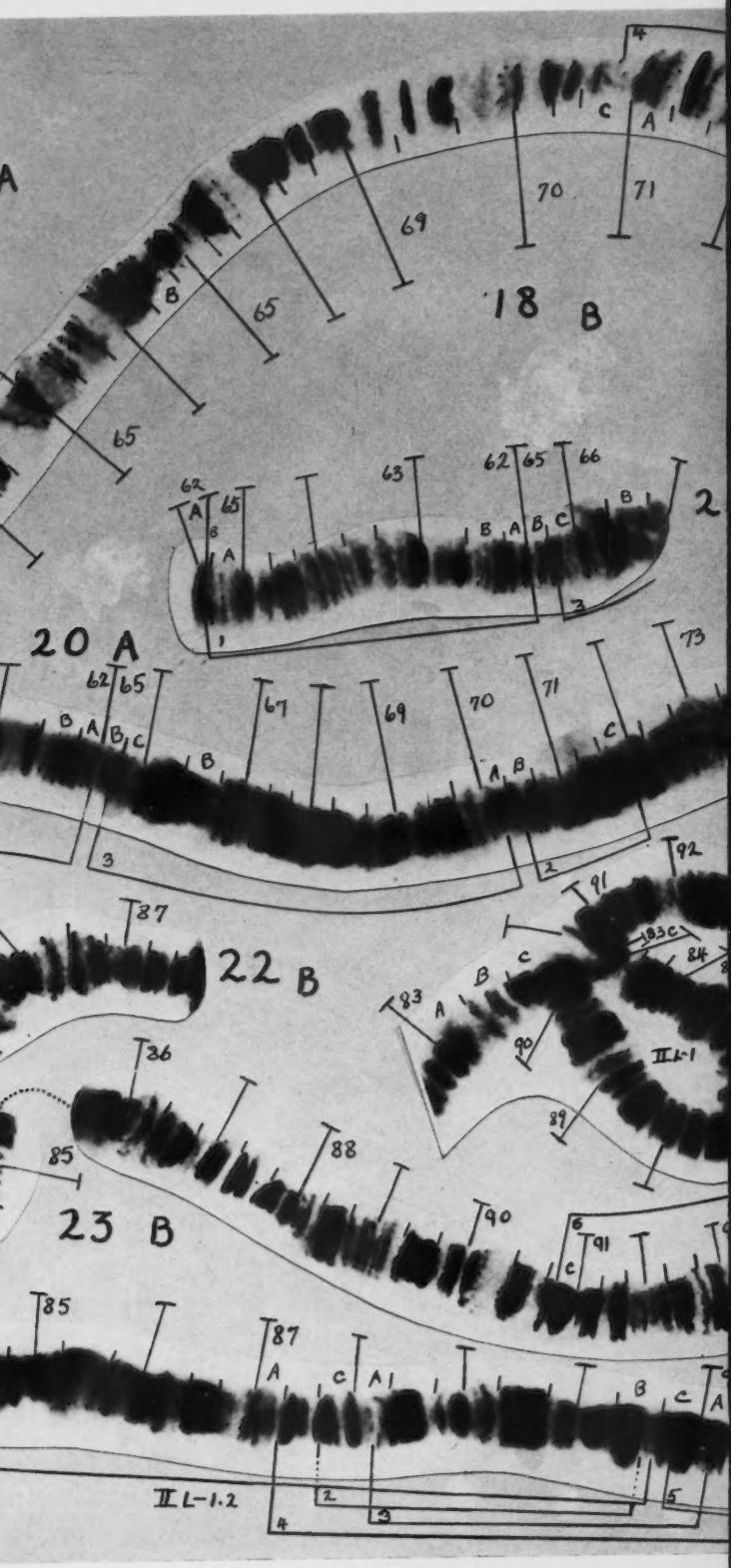
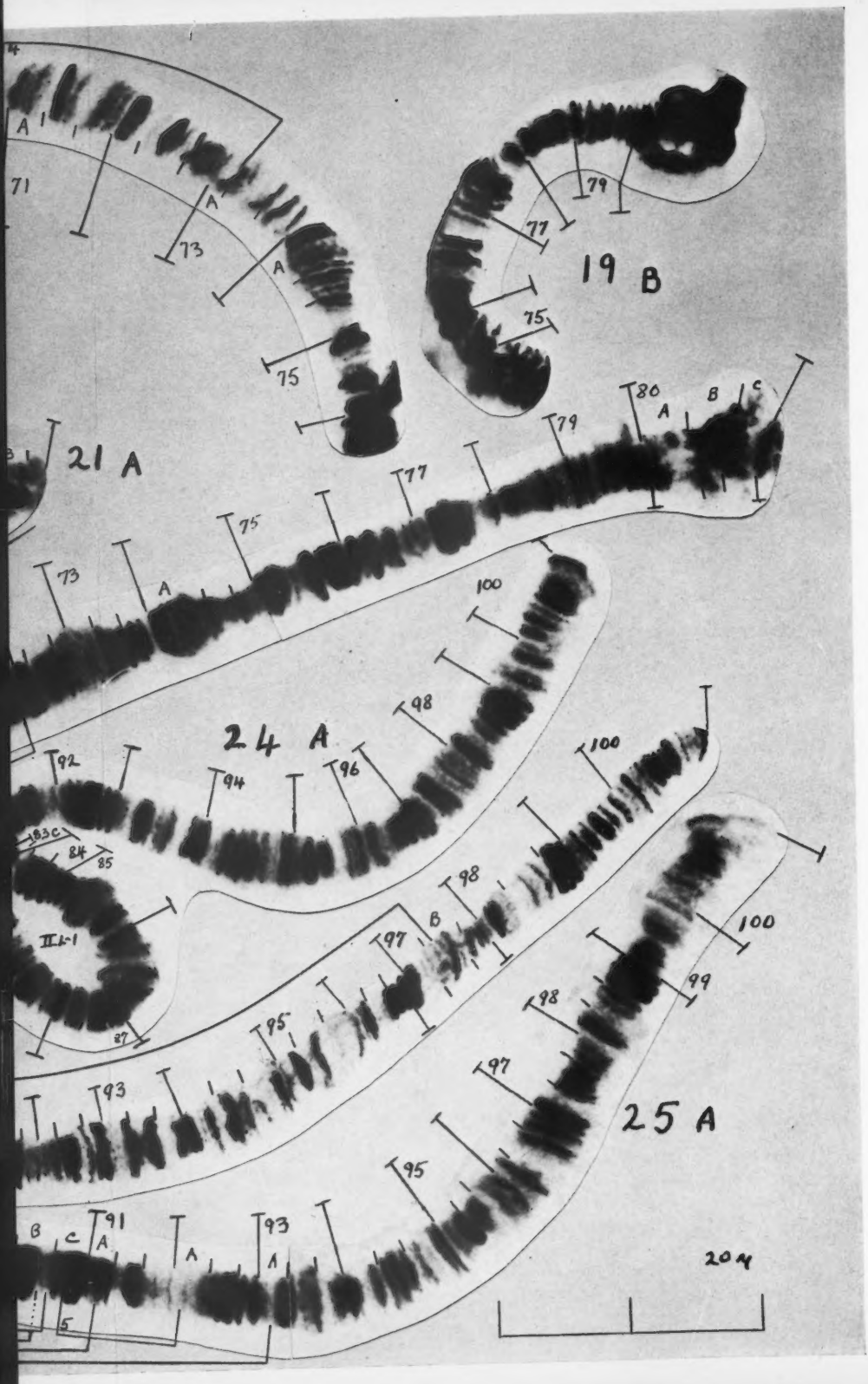
53
51
49
47
45
59
57
IL-2.9


13 A
27
10
8
IS-4
7
6
C
B
A
12
13
A
B
C
55
56
A
C
B
59
58
B
C
C
56
59
C
B
60
A
61
2
1
B
C
A
C
59
60
61
IL-2.9
204
B
57
56
60
59
C
61


17 A
II S-1
64
62
A
B
65
B
A
65
C
66
C
3
A
62
65
62
63
68
20
62
B
A
B
A
65
63
62
1
3
81
82
85
81
83
85
84
23
81
83
85
C
1


18 B
20 A
22 B
23 B
II L-1
II L-1.2


19 B
21 A
24 A
25 A
71
73
75
77
79
80
A
B
C
100
98
92
94
96
83C
84
85
87
97
95
93
91
99
20 4

in southern Ontario, and the overlapping inversion IL–2.9 is a rare alternative sequence in A. Since the two sequences in A are phylogenetically related through *IL-2* of B it is clear that this sequence has existed at least at one time also in A. There is the remote possibility it may yet turn up in southern Ontario in A (46, 47). For the present *IL-2* is shown in the maps and idiogram as an interspecific inversion and IL–2.9 as a floating inversion A. In actuality any B chromosome differs from either A sequence by a single inversion step (Fig. 9*b*). As in IS, no floating inversions were found in IL in B; thus their presence in the long chromosome so far identifies sibling A.

Apart from IL–2.9 all floating inversions in this arm are simple in respect to standard. Only IL–3 is common, represented in 32% of the constituents; IL–4 is in 8%. The remaining sequences IL–5, IL–6, IL–7, IL–8, and IL–2.9 occur with frequencies of 3% or less. IL–3 to IL–8 are so distributed that each overlaps from one to three other inversions on the map. No overlapping heterozygous configurations were found in this region of the chromosome except for a single individual heterozygous for IL–3 and IL–8 (which also had IL–5 and IL–2.9 heterozygously). IL–3 was found at times accompanied by either IL–4, IL–5, or IL–6. IL–2.9 in both instances of its occurrence was accompanied by IL–3.

Second Chromosome, Short Arm, IIS

The second chromosome is divided into two nearly equal parts by the dark band of the expanded region. In clear preparations the statistically shorter arm is identified by the presence of the nucleolus at the base of the arm very close to the expanded region. An additional landmark of some use is the characteristic configuration (Figs. 18, 20: section 74A) which suggests somewhat a "cup and saucer". This section is particularly interesting since a similar grouping has been found in the short arm of the first (long) chromosome of all species of *Prosimulium* studied by Rothfels (28). Finally it is possible to use known inversions, particularly the heterozygous configurations, to identify this arm and also to differentiate the two siblings.

There are no interspecific differences in this chromosome so that sibling B has the same arrangement as the standard A. But in the shorter arm sibling A has one inversion IIS–1 that occurs in 91% of the constituents (Figs. 17, 20, 21; Table IV). The standard identical with the B arrangement occurred so infrequently in our sample, and only once homozygously, that for all practical purposes IIS–1 may be considered interspecific and thus may supplement the distinction of siblings A and B. On the maps Figs. 20 and 21 represent the "common" arrangement for A whereas Fig. 18 of B represents the standard sequence.

FIGS. 17–21. Chromosome II, short arm, IIS.
FIG. 17. Sibling A, heterozygous for inversion IIS–1. FIGS. 18, 19. Sibling B, standard band sequence for both siblings. FIGS. 20, 21. Sibling A, common band sequence, homozygous for inversion IIS–1.
FIGS. 22–25. Chromosome II, long arm, IIL.
FIGS. 22, 23. Sibling B, standard band sequence. FIG. 24. Sibling B, heterozygous for inversion IIL–1. FIG. 25. Sibling A, standard band sequence.

There is in A one rather uncommon inversion, IIS-2 (Table IV) appearing in 8% of the constituents and randomly associating with IIS-1. The single constituent with IIS–3 did not have the common inversion IIS–1 although the other constituent did have IIS–1, forming a double loop distally. IIS–4, one of the two inversions found in sibling B occurs in this arm. Four specimens with it came from the same collection (Holland Marsh, Sept. 30, 1956), so quite conceivably they were sibs (Table IV).

In sibling A an additional small discrepancy was noted in clear preparations in section 66B (Fig. 5) of some slides which was considered as a possible cytological sex determining mechanism. In Fig. 6 the constituent marked "X" is the standard while the side marked "Y" is the possible sex determining variation. Of 26 slides where the heterozygous condition was observed five were not sexed, 20 were male, but one was labelled female. On the other hand, in any slides clear enough to be checked, the "Y" variation was never observed homozygously. Unfortunately there were two difficulties encountered in checking this character. The one slide marked female may have been mis-sexed because of the difference in distinguishing the gonads in immature larvae. The second difficulty was that most of the preparations were too poor to resolve the detail in 66B. Sibling B had no comparable structure in its complement. The evidence for a cytological sex determining mechanism in *E. "aureum"* is not as conclusive as for other black flies (27, 28, 50).

Second Chromosome, Long Arm, IIL

This arm may be identified in that it lacks the diagnostic features of IIS. In addition, a series of four band groups (Figs. 23–25: 90B–91B) has been found to be of some use as a positive marker, and an aid in sorting the various floating inversions.

Except for the characteristic floating inversions there is no difference between siblings A and B in this arm. The most common inversion in this arm in A, IIL–1, occurs in 25% of the constituents. IIL–2 was found heterozygously, always included within the limits of IIL–1 (IIL–1.2, Table IV). IIL–1.2 was found paired twice with IIL–1, and eight times with the standard producing a lopsided figure-of-eight. The other three inversions in A, IIL–3, IIL–4, and IIL–5, which are overlapping or included on the map have only been found singly heterozygous. None of them are found in more than 3% of the constituents (Figs. 8, 23–25; Table IV). IIL–3 and IIL–4 did form overlapping loops with IIL–1, five times for IIL–3 and once for IIL–4.

The second floating inversion in B, IIL–6, is in this arm. It was found only twice heterozygously and again both individuals came from one sample (Holland Marsh, Aug. 18, 1956). Although four IIS–4 heterozygotes came from the same stream, they were not collected on the same date.

Statistics

The four most common inversions in sibling A were tested for conformity with the Hardy-Weinberg distribution. In the 191 larvae that could be completely classified (Table IV), accepting $p = .05$ as the level of significance,

IS–4, IIS–1, IIL–1 can be assumed to be randomly distributed but p for IL–3 is .004 (Table V). This discrepancy for IL–3 is due to there being more heterozygotes than expected. As a further test, regional subsamples were compared individually with the Hardy-Weinberg distribution. In all subsamples it was found that heterozygotes exceeded expectation, but the numbers of individuals in the subsamples were too low to make a statistical analysis useful.

Three by three contingency tables were used to test for association between inversions IS–4, IL–3, and IIL–1. IS–4 × IL–3 gave $p = .2$, IS–4 × IIL–1 gave $p = .3$, but IL–3 × IIL–1 gave .02 (table VI). Since two of the cells of this last pair (S/S × 1/1 and S/3 × 1/1) account for 70% of the chi-square value and represent only 4% of the larvae this value for p may be ascribed to chance. There is no decisive evidence of association between any of these inversions; they apparently associate randomly.

TABLE V

TEST FOR CONFORMITY WITH THE HARDY-WEINBERG DISTRIBUTION OF IS–4, IL–3, IIS–1, AND IIL–1

	IS–4			IL–3			IIS–1			IIL–1		
Combination	S/S	S/4	4/4	S/S	S/3	3/3	S/S	S/1	L/1	S/S	S/1	1/1
Observed	46	88	57	79	101	11	1	33	157	106	75	10
Expected	42.4	95.2	53.4	87.7	83.5	19.8	1.6	31.8	157.6	107.9	71.3	11.8
χ^2		1.15			8.44			0.24			0.52	
p		.3			.004			.7			.5	

TABLE VI

TEST OF ASSOCIATION BETWEEN IS–4 AND IL–3, IS–4 AND IL–1, AND IL–3 AND IL–1

	IS–4 × IL–3				IS–4 × IIL–1				IL–3 × IIL–1			
	S/S	S/4	4/4		S/S	S/4	4/4		S/S	S/3	3/3	
S/S	20 * 19.0	35 36.4	24 23.6	S/S	23 25.5	48 48.8	35 31.7	S/S	42 43.8	29 31.1	8 4.1	
S/3	21 24.3	47 46.6	33 30.1	S/1	20 18	33 34.6	22 22.4	S/1	60 56.1	40 39.6	1 5.3	
3/3	5 2.7	6 5.0	3.3	1/1	3 2.5	7 4.6	2.9	1/1	4 6.1	6 4.3	1 .6	
	$\chi^2 = 6.31$ $p = .2$				$\chi^2 = 5.00$ $p = .3$				$\chi^2 = 10.3$ $p = .02$			

*Figures in the upper left represent the observed frequency; in the lower right, the expected frequency.

The Distribution of Break Points

A glance at the idiogram (Fig. 8) will show that no one arm in sibling A is favored for inversions, unlike the condition found in other Diptera (12, 30). IS has three floating inversions and three interspecific ones; IL has seven floating and two interspecific inversions; IIS and IIL have three and five floating inversions respectively. One of the four common inversions is found in each arm (Table IV).

In IS the 12 break points are concentrated towards the distal end. Only the break points for *IS–1* distal and IS–4 proximal are nearly coincident, but in view of the relatively large number of breaks in this arm no significance is attached to these two specific breaks.

Fourteen of the 18 breaks of IL are concentrated centrally where the distribution of inversions shows a striking, regular pattern characterized by serially aligned tandem or near tandem inversions forming a tandem succession. For example IL–7 fits between IL–3 and IL–5 while IL–5 in turn includes IL–4 and IL–6; IL–8 and *IL–1* are the start of a similar pattern. These patterns entail considerable break coincidence in four regions: IL–5 distal and IL–6 proximal; IL–4 and IL–5 distal (coincident), and IL–7; IL–7 distal and IL–3 proximal; and IL–8 proximal and *IL–1* distal.

In IIS the tandem succession is equally evident in the succession if the three inversions in sibling A in which the breaks between IIS–1 proximal and IIS–3 distal and IIS–3 proximal and IIS–2 distal are separated by only three or four bands.

The tandem pattern is again observed in IIL in the near coincidence of breaks of IIL–1, IIL–2 plus IIL–3, and IIL–5 plus IIL–6 all in section 90B to 91A; also of IIL–2, IIL–3, and IIL–4 in section 87B–88A.

The frequency of tandem and near tandem inversions and the occurrence of coincident and near coincident breaks which this entails follows the pattern observed for chironomids by Rothfels and Fairlie (30). These authors invoke break induction by heterozygous inversions as the cause of the apparent nonrandomness of chromosome breaks. There is no evidence that any multiple break rearrangements occurred since with all triads the three possible arrangements are known.

Other Siblings

Of the 11 preparations obtained from southern Quebec (Table I) four were sibling A, with six of the more common floating inversions found in the Toronto area, IS–4, IL–3, IIS–1, IIL–1, IIL–2, and IIL–3 plus one small new one, IS–6. The other seven were type B with no inversions. From this similarity between "*aureum*" siblings in the Montreal and Toronto areas it was assumed that in the territory between north of the St. Lawrence River and Lake Ontario, the same two sympatric forms are found. From Stone and Jamnback's larval description (40) both forms are apparently present south of this area in New York State as well.

In the summers of 1951, 1953, 1955, and 1956 preparations of two additional siblings of *E. "aureum"* among others were made at Fort Churchill, Manitoba. Mature larvae of sibling C were collected in August from a cold, coastal tundra trickle not more than 75 yards long and seemingly supplied mainly by melting permafrost. Larvae of sibling D were found from late June to the end of July in a warm creek 12 miles inland in a fairly densely forested area. These forms were tentatively compared with the two Toronto ones. The entire second chromosome and the long arm of the first chromosome of sibling C were found to compare with sibling B; while the short arm was found to have the

same arrangement as A for *IS–3*, and the missing arrangement intermediate between A and B for *IS–1* and *IS–2* (Figs. 8, 9*a*). The forest form, sibling D, compares with the tundra form in the entire second chromosome and the short arm of the first chromosome but in the long arm there is a new, as yet unresolved, basic arrangement. Sibling C is somewhat like B in the paucity of floating inversions, only one heterozygous loop being recorded in 46 specimens. None of the floating inversions in D are like the one in C, and both C's and D's are unlike any of A's or B's floating inversions.

In 1955, 12 slides were prepared from material received from the Campus of U.C.L.A., Berkeley, California (2). The commonest homozygous condition was not worked out, but it is clearly unlike that of any of the above four siblings. The floating inversions also were different again from any known previously.

The gular cleft of siblings C, D, and of Californian and Alaskan material (38) is similar in shape to that of sibling B. The minute secondary characters about the gular cleft noted for A and B (Fig. 1) were also examined and differences found which may yet prove useful in separating morphologically the siblings so far looked at. No detailed examination of any of the above characters was undertaken. On the other hand the gular cleft in Grenier's illustration of *E. aureum* from France is distinctly different from anything mentioned above (15). In all probability this represents a sibling different from any on this continent.

The results of other work done in this laboratory show such a divergence of form of a single species to be fairly common among black flies. Rothfels reported two siblings of *Prosimulium "hirtipes", 1* and *2*, from Ontario (27). A third sibling from Ontario, one from New York, and several from Europe have been distinguished (28). Among other black flies with several siblings indicated from cytological evidence are *E. "latipes"* and *S. "tuberosum"* (49, 50). Similar diverging groups of sibling species based on cytological evidence have been reported for *Drosophila* (5, 19, 21, 40).

Origin of the Dichromosomic Condition

The dichromosomic condition of *E. "aureum"* siblings in all probability developed from the trichromosomic conditions found in all other black flies so far examined. To achieve this it is suggested that the two smaller chromosomes, II and III of other simuliids, "fused" to form the first or long chromosome of *E. "aureum"*. A possible mechanism would be the formation of two acrocentric chromosomes, by pericentric inversions or centromere shifts, followed by an unequal interchange which may have given rise to a dicentric first chromosome and a small accentric fragment which was subsequently lost.

Support for this is obtained from a comparison of sections of the banding pattern of *E. "aureum"* with that of *Prosimulium* and an unidentified and probably unnamed *Eusimulium* which was used because it has the same very tight pairing as *"aureum"*. The segment 19A to 21C, including the three heavy bands of section 20 from IS of *E. "aureum"* (Figs. 10–12), was found in the R.o.B. bearing shorter chromosome of the unidentified *Eusimulium*. The

other short chromosome had a region which compared with section 59 to 54, and possibly section 54 to 45 as well (Figs. 14–16), in IL of *E. "aureum"*. Thus there are band regions from the two short chromosomes, II and III, of a three chromosomed *Eusimulium* found combined in the long chromosome, I, of *E. "aureum"*. Finally there is the "cup" and "saucer" (74A) region, 73B to 75C (Figs. 18, 20), in IIS of *E. "aureum"*. This region is typical of the IS arm of species of *Prosimulium* (28).

Further, the nucleolus is found near the expanded region in the second chromosome of *E. "aureum"* whereas in several *Eusimulium* and *Prosimulium* it is also near the centromere but in the first chromosome. The R.o.B. in all trichromosomic black flies is in one of the shorter chromosomes; in "aureum" it is in the longer. Finally, the % TCL of the second chromosome of "aureum", 39% compares closely with that of the first chromosome of all *Simulium* and *Eusimulium* (39–42%) (50), and all *Prosimulium* (40–47%) (28), so far checked in this laboratory. But the two arms of this chromosome do not compare so closely; in *"aureum"* they are nearly equal, 19% and 20%; in *Eusimulium*, 18% and 24%; in *Simulium*, 19% and 22%; and in *Prosimulium*, 20% and 25%. The % TCL of the two arms of the first chromosome of *E. "aureum"*, 25% and 36%, does not compare well with % TCL of II and III of *Simulium* and *Eusimulium*, 29% and 30%, or of *Prosimulium*, 30% and 26%. This discrepancy in arm ratios of I of *"aureum"* compared with ratio of II to III of other black flies does not necessarily invalidate the hypothesis.

Discussion

The first eight slides of *E. "aureum"* salivary gland chromosomes from the Toronto area in 1952 could be readily separated into two groups on cytological grounds. That summer it was found that the basic arrangement of the group lacking floating inversions differed from the other group by five homozygously inverted regions, *IS–1.2*, *IS–3*, *IL–1*, *IL–2*, and IIS–1. It was suggested then that there were two distinct but closely related species, subsequently distinguished as *E. "aureum"* siblings A (with the floating inversions) and B. During the next 2 years 18 type A and 31 type B (Table I) were examined with the resulting discovery of four new floating inversions in sibling A. It became apparent from these collections that the two siblings occurred together in the same stream at the same time.

Finally, to consolidate the claim to the existence of two species, 587 more larvae from the Toronto area were examined cytologically in 1956. Of these 189 were sibling A and 398 were sibling B. In spite of all this additional data the original observations were essentially unchanged; only two modifications were noted. First IIS–1, originally considered an interspecific inversion, was shifted to intraspecific rank with the discovery of the B arrangement (now standard) in A (Table IV). The second change was discovery of the floating inversion IL–2.9 in A. Since this complex is the third member of a triad (Fig. 9*b*) with standard B (*IL–2*) intermediate, *IL–9* can be considered as interspecific as mentioned in the description of IL. There were also recorded eight new floating inversions in A, and the only two so far found in B.

To sum up, 644 larvae of *E. "aureum"* siblings from the Toronto area were examined cytologically, 209 of which were sibling A and 435 were sibling B. Both siblings compare band for band throughout the two chromosomes but they differ by four homozygously rearranged regions all in the long chromosome, including inversions *IS–1.2*, *IS–3*, *IL–1*, and *IL–2* (plus IL–2.9), (Figs. 8, 9*a*, 9*b*, 10–16).

Sibling A has considerable chromosomal polymorphism, 18 floating inversions being known for populations from southern Ontario and Quebec (Tables I, IV). All but 12 larvae of A had from one to six heterozygous inversion loops. Of the 12 exceptional larvae only three were homozygous for the "common" arrangement (standard except for IIS–1), and none for standard. In sibling B on the other hand only two inversions, IIS–4 and IIS–6, were found as heterozygous loops, the first five times and the second twice.

A possible sex determining mechanism was found in section 66B of sibling A, but because of poor preparations this could not be positively checked. There were no such differential segments in sibling B.

In the area from which the two forms checked cytologically were collected, they are completely sympatric, having been found together throughout the entire season, from May to October, in any stream from which a sample of eight or more was obtained (Table I). From this it appears there are no ecological or seasonal barriers to prevent crossbreeding. This suggests other barriers such as generic or physiological factors (21). Unfortunately black flies are not amenable to breeding in captivity so possible mating experiments with laboratory colonies are not feasible (8, 9).

Should interbreeding occur between these two siblings there would be two prominent cytological conditions found to indicate such matings. First, there would be a swamping of sibling B's spectrum of floating inversions with A's inversions and vice versa to give common floating inversions. Secondly, heterozygous inversion configurations would be expected at all four locations of interspecific inversions, possibly complicated by floating inversions. Since neither critical interspecific heterozygotes nor common floating inversions were found, the cytological evidence indicates that *E. "aureum"* siblings A and B are two biologically distinct species.

Summary

A band for band comparison of the salivary gland chromosomes of the first eight preparations of the dichromosomic black fly *E. "aureum"* in 1952 showed two larvae (with seven floating inversions, sibling A) to differ from the other six larvae (with no floating inversions, sibling B) by five homozygously rearranged regions; inversions *IS–1.2*, *IS–3*, *IL–1*, *IL–2*, and IIS–1. Subsequent cytological examination of all 644 preparations of both siblings collected from seven Toronto area localities did not essentially change the original observation. The results showed (1) in 209 slides of sibling A, 18 floating inversions are recorded, four of which are common (Table IV); (2) two rare floating inversions are found in 435 slides of sibling B in the shorter chromosome (IIS–5,

IIL–6, Table IV); (3) the number of interspecific homozygously rearranged regions was reduced to four with the finding of IIS–1 occasionally paired with the arrangement in B; (4) the rare floating inversion IL–2.9 in A is an alternate specific inversion, considering the absence of the B arrangement (*IL–2*) in A (Fig. 9*b*).

The only taxonomic character recorded to separate the two siblings is the shape of the gular cleft in the larva. Adults and pupae were collected but not examined.

To provide a basis for descriptive work photographic maps of the entire salivary gland complement of both siblings were prepared (Plates II and III). Because of the remarkable similarity in gross features the general description of the giant chromosomes applies to both siblings.

The % TCL for the salivary gland chromosomes of both siblings (Table III) is presented graphically in the idiogram (Fig. 8).

Because of approximately the same arm ratios about the expanded regions and the mitotic centromeres these two regions are homologized. Within the expanded regions are heavy bands assumed to be centromeres by comparison with centromeres proved in other black flies. In the first chromosome, the two heavy bands (24C and 27A) in the seemingly longer than usual tufted portion of the expanded region are both suggested to be centromeres. Thus the first chromosome of *E. "aureum"* is dicentric.

The four common inversions were tested for association (Table VI) and found to be associating randomly. In testing with the Hardy-Weinberg distribution (Table V), IIS–3 was the only inversion that did not fit; there was insufficient data to determine the reason.

The regular tandem pattern produced by the non-random distribution of the inversion break points is suggested to be a result of break induction by heterozygous inversions.

Siblings A and B are so far found sympatric throughout their known territory, which includes southern Ontario and Quebec and New York State. Two other cytological siblings have been recorded from Churchill, Manitoba, and one from Berkeley, California. The implications are that there are more siblings to be found through *E. "aureum"*'s holarctic range.

The origin of the two chromosome condition of the five known cytological siblings is suggested to have arisen from the fusion of the two small chromosomes of the normal three chromosome condition in black flies by an unequal arm interchange to form the long chromosome of *"aureum"*. Comparisons of sections of the banding patterns, locations of the R.o.B. and nucleolus, and % TCL similarities between *"aureum"* and other black flies are presented as evidence.

Finally, if these two sympatric sibling species were to interbreed, then common floating inversion and heterozygous loops formed by the interspecific inversions should be expected. Since neither of the above two conditions were found, *E. "aureum"* siblings A and B are considered distinct biological species.

Acknowledgments

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STUDIES ON THE DEVELOPMENT OF THE KIDNEY OF THE PACIFIC PINK SALMON (ONCHORYNCHUS GORBUSCHA (WALBAUM))

II. VARIATION IN GLOMERULAR COUNT OF THE KIDNEY OF THE PACIFIC PINK SALMON[1]

PETER FORD

Abstract

Counts of the glomeruli of pink salmon fry raised in fresh water were compared with those of fry raised in sea water. A significant increase in the count was observed in fry of the same fork length raised in fresh water.

Introduction

The publications of Smith (4, 5) concerned with the relationship of glomerular apparatus to adaptation to osmotic habitat are too well known to discuss exhaustively. He has been able to correlate the phylogeny of the vertebrates with the elaboration of the filtration apparatus and postulates that the colonization of fresh water was possible by reason of the introduction into the protovertebrate nephron of a glomerular ultrafilter capable of removing large volumes of water and dialyzable solutes from the blood. The subsequent return to salt water required the reduction of this filter to offset water lost by exosmosis. In the elasmobranchs this was possible through the evolution of the urea-retention habit. In the bony fish this was accomplished either by reduction of the number of glomeruli, the reduction of glomerular vascularity, or the closure of the neck segments of the nephrons.

It was hoped that a study of the kidney of a migratory fish from the time of its normal seaward journey might shed some light on the effects of osmotic environment with respect to the development of glomeruli in a single species. The short 2 year life cycle of the pink salmon makes it a suitable animal for such a developmental study.

Materials and Methods

Eggs stripped from individuals were raised in the hatchery and maintained until normal migration time (7 months after hatching) when the population was halved, one group being transferred to sea water and the other retained in fresh water.

The two series were sampled at weekly intervals, fixed, stained, and mounted. Sections were cut at 20 μ and the glomeruli counted.

Results

Glomerular counts (Table I) reveal an apparently larger number of glomeruli in fresh water fish than in sea water specimens of a given fork length (Fig. 1).

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Contribution from the Department of Zoology, University of British Columbia, Vancouver, British Columbia. This study was supported by a grant in aid of research from the National Research Council of Canada.

TABLE I

Date	Fresh water series		Sea water series	
	F. length	Glomeruli	F. length	Glomeruli
4/6	32.0	177	37.0	213
	36.2	291	35.1	194
	33.4	194	35.2	176
	33.4	263	36.0	181
	27.7	135	31.0	146
	30.9	221	29.6	129
	27.8	249	30.2	153
	32.1	217		
18/6	37.5	320	43.7	310
	33.5	268	39.8	279
	32.8	219	40.2	286
			37.6	230
25/6	38.6	325	39.8	251
	37.4	310	34.4	208
			34.0	248
			33.4	219
2/7	36.9	303	45.8	307
	38.7	342	41.7	288
	33.1	237	44.6	293
	33.9	286	32.3	204
9/7	40.8	332	54.9	576
	36.7	295	51.7	376
	33.6	253	36.0	229
	29.3	164	36.6	277

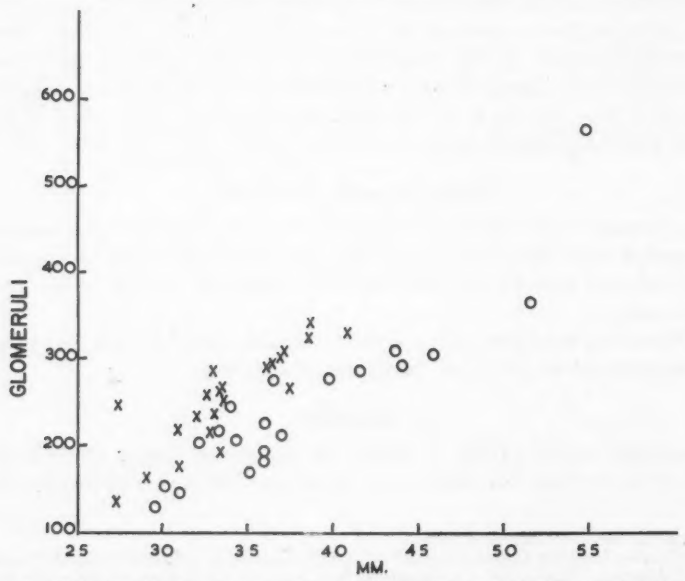

FIG. 1. Relationship between number of glomeruli and fork length of pink salmon maintained in fresh water (×) and in sea water (○).

Analysis of covariance of the arithmetic data (Table II) indicates that the two sets of values differ in a highly significant manner, the fresh-water fish having a larger number of glomeruli.

TABLE II

Source of variation	Degree of freedom	Sum of squares	Mean square
Total	40	66,830.86	—
Treatments	1	34,781.36	34,781.36
Error	39	32,049.50	821.78

NOTE: F (variance ratio) = 42.32.
P (probability) < .01.

Discussion

The results obtained in these experiments indicate that fresh-water forms of fish when subjected to salt water environment reduce the number of glomeruli and in so doing reduce the rate of filtration of the kidney as a whole. This result bears out the observation of Smith (4), who states "marine fishes are under continuous selection pressure to reduce or even obliterate the glomeruli."

Summary

1. The scatter diagram (Fig. 1) shows the relation of the fork length and number of glomeruli for each group are different.

2. To convenience analysis the two very high values were not used. This is desirable since these two high values bias comparison of the two sets of points which cover, with these exceptions, a similar range.

3. The two sets of points were compared by analysis of covariance. The fresh water group have a larger number of glomeruli and the difference is highly significant.

4. These results are in agreement with those postulated by Marshall (2) and Smith (4), who both concluded that marine fish tend to have fewer glomeruli than fresh water fish.

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THE EFFECT OF TEMPERATURE ON THE SPONTANEOUS ACTIVITY OF SPECKLED TROUT BEFORE AND AFTER VARIOUS LESIONS OF THE BRAIN[1]

KENNETH C. FISHER AND CHARLOTTE M. SULLIVAN

Abstract

The frequency of spontaneous movements made by speckled trout was determined at a number of different constant temperatures. The relation between the frequency and temperature was found to be complex, showing two maxima. One of these constantly occurred at approximately the temperature selected by normal trout in a horizontal temperature gradient and the other occurred two to three degrees below the upper lethal temperature.

The relation between frequency of movements and temperature was not affected by (1) destruction of the forebrain, (2) by section of the lateral line nerves, or (3) by destruction of *both* the forebrain and the dorsal part of the cerebellum. Destruction of *only* the dorsal part of the cerebellum profoundly changed the relation between frequency of movements and temperature: it caused the disappearance of the first peak.

Brief consideration was given to the legitimacy of relating the activity of animals in different constant levels of environmental factors with behavior in gradients of these factors.

Introduction

Even in an environment where all conditions are constant most, if not all, animals make movements from time to time. Such movements are usually called "spontaneous" because the initiating stimulus is not known.

In most natural environments animals are subject to regular fluctuations of environmental factors and in many cases activity of animals is related to these fluctuations. Thus the occurrence of regular periods of activity in some animals is related to the daily light cycle — the animals are diurnal or nocturnal (Gunn, 1940 (16); Bentley *et al.*, 1941–42 (2); Spoor, 1946 (32)). Rao (1954 (28)) found that activity of *Mytilus* is in phase with the tidal cycle. Activity occurring thus regularly can, moreover, in many instances, be shown to be truly "spontaneous" in that in the absence of the usual change in environmental conditions the activity rhythm persists for a longer or shorter time (Gunn, 1940 (16); Bentley *et al.*, 1941–42 (2); Rao, 1954 (28)).

The amount of activity, as measured by the number of animals active at any given moment, during either the active or quiescent phase of the activity cycle, has been shown to be affected by environmental factors other than the regularly fluctuating one. Thus Bentley *et al.* (1941–42 (2)), studying *Ptinus tectus*, which is nocturnal, found that the number of animals active at any moment was greater at high than at low humidity in both the dark and light phases of the daily light cycle; that more animals were active at low than at high temperature during the dark part of the cycle; and that there was greater activity at low than at high temperature in constant light. Several investigators have shown that there is a relation between numbers of animals active at a given instant, and the humidity of the environment

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Contribution from Department of Zoology, University of Toronto, Toronto, Ontario.

(for example, Gunn, 1937 (15); Gunn and Pielou, 1940 (19); Bentley, 1944 (1); Bursell and Ewer, 1950 (4)). The number of organisms in motion may be taken as an index of the frequency with which movements are made by individuals and these movements are "spontaneous" in the sense that each did not result, in so far as the observers knew, from some particular change in the physical or chemical conditions of the environment.

The relation of frequency of movements to different levels of various factors has been regarded by Fraenkel and Gunn (1940 (11)) as of particular interest in connection with the phenomenon of aggregation or selection. If frequency of movements varies with the intensity or level of some factor, then in an environment which is non-uniform with respect to this factor selection of, or aggregation in, a particular part of the environment might occur as a result if other things were equal. Gunn (1937 (15)) concluded, for example, that wood lice aggregated in the more humid side of an alternative humidity chamber because they were less active in moist than in dry air. Other workers (Gunn and Pielou, 1940 (19); Waloff, 1941 (36); Bursell and Ewer, 1950 (4)) have concluded that similar differential effects of humidity on activity contributed more or less to the mechanisms of aggregation of their experimental animals in one or the other side of alternative humidity chambers.

In this laboratory there has been an interest in the mechanisms by which organisms aggregate in or select a particular part of an extended horizontal temperature gradient (Fisher and Elson, 1950 (10); Smith and Fisher, 1956 (31)). There are, of course, many instances in which activity of animals in relation to temperature has been recorded (e.g. Crozier and Stier, 1925–26 (6); Miller, 1929 (26); Nicholson, 1934 (27); Gunn and Hopf, 1941–42 (18)). Recently Chapman (1955 (5)) measured velocity of walking of locust nymphs in different parts of a gradient of temperature and concluded that there was some correlation between low velocity and selected temperature. None of these studies has, however, given any clear proof that effects of temperature on the activity recorded account for, or even play any part in, the mechanism of temperature selection. In short it appears that the mechanism of temperature selection has not yet been satisfactorily described for any animal.

The present investigation, which deals with the effect of temperature on the frequency of spontaneous movements made by fish, is therefore of interest for its possible connection with the mechanism of temperature selection (well-established for fish), as well as for its own sake, as one of the fundamental effects of environmental conditions on activity. Frequency of spontaneous movements of trout was measured at each of a number of temperatures. Experimental animals were equilibrated to each test temperature by being exposed to it for at least fifteen minutes before movements were counted. This was sufficient time for body temperature to become the same as the environmental temperature (Gunn, 1942 (17)) but the fish were in no sense acclimated to it since the process of acclimation to a new environmental temperature requires a considerably longer period (Fry, 1947 (12)); Bullock, 1955 (3)).

Materials, Apparatus, and Methods

Young speckled trout, *Salvelinus fontinalis* (Mitchill), 2 to 3 in. long, were used in the experiments. These fish were obtained from a private hatchery and held in running Toronto tap water.

The chamber (Fig. 1*a*) in which each fish was placed for observation was made of aluminum and was painted flat white inside. Nine inches high and with a diameter of 6 in., its capacity was about four liters. This fish chamber was set in a water bath (*b*) which was equipped with heater and stirrer, inlet, and constant level outlet device.

Fresh water entered the chamber containing the fish continually, at a rate of about one-quarter liter per minute. This water from the city supply was brought to the temperature of the outside bath by passage through a coil of aluminum tubing immersed in the bath. It entered the fish chamber at the bottom. Overflow from the fish chamber ran over its edges into the bath. A plate glass lid (*c*) on the chamber prevented the fish from jumping out.

Typically, the constant temperature bath was supplied continuously with cold water from the city supply and was kept at the actual temperature desired by electric heaters controlled by a thermoregulator–relay circuit, the

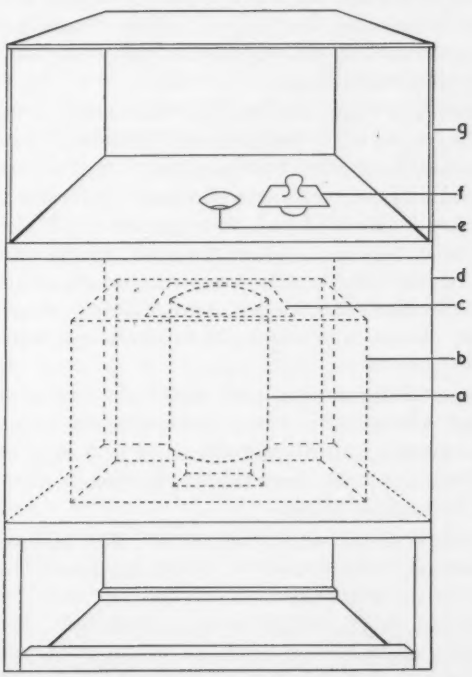

FIG. 1. A single fish was observed in container *a* which was kept at a constant temperature by immersion in the bath *b*. A slow flow of water entered *a* continuously, overflowing under the glass cover *c*. Bath *b* was totally enclosed except for the observation port *e*. For further details see text.

temperature sensitive element of which was a resistance thermometer. The use of the latter made possible precise and rapid changes in the temperature setting of the bath. In summer, when the temperature of the tap water was above that desired in the bath, control at lower temperatures was achieved by using a pump in place of the heater in the relay circuit. The pump was brought into operation by the thermoregulator and moved water from the bath into a large can filled with ice, whence it flowed back into the bath by gravity. Differences in temperature between the bath and the chamber were detected by a thermocouple, one junction of which was in the fish chamber, the other in the bath.

The fish chamber and constant temperature bath were housed in a light-tight box (Fig. 1*d*). A hole (*e*) cut in the top of this box was kept covered by a light-tight lid (*f*) except when observations were being made through it. A black beaverboard cowl (*g*), open at the front was over the box. It was spacious enough to admit the head and shoulders of the observer. Light from the room was excluded by draping a black cloth over the cowl and the observer.

Observation of the fish was made possible by four flashlight bulbs, one at each corner of the constant temperature bath. These were placed below the edge of the fish chamber so that they did not illuminate the animal directly. Light was kept constant and was adjusted to the minimum intensity which permitted observations to be made.

Each experiment was begun by bringing the water of the bath and the fish chamber to the desired initial temperature. One trout was then introduced into the fish container and left for 20 minutes to half an hour before observations were begun. When an observation was to be made the experimenter thrust his shoulders under the cowl, drawing the black cloth over himself. He removed the lid so that he could see the fish; then he released an interval timer placed at his feet and with a hand counter recorded the number of movements made by the experimental animal over a given period of time, usually 5 minutes. Before withdrawing from his observation post the observer replaced the lid.

Temperature was changed after each reading. Temperature equilibrium between bath and fish chamber could be established at a new level in 10 minutes, using a mixture of hot and cold water to hasten the change in the bath. The fish was allowed at least fifteen minutes at the new temperature before the next reading was taken.

Experiments were done on normal fish, on fish with lesions of the forebrain and of the cerebellum, and on trout in which the lateral line branch of the vagus nerve was cut on both sides.

It was considered desirable to make these operations on the central nervous system as simply as possible. Accordingly the operations on both forebrain and cerebellum were done without opening the cranium extensively. To accomplish forebrain destruction a fish was lightly anesthetized in water saturated with ether. The animal was wrapped in a piece of damp cheese

cloth with the head projecting. By holding the fish's mouth open and allowing light to shine on the roof of the mouth, the outline of the brain could be clearly seen through the dorsal surface of the brain capsule. At a point between the anterior ends of the optic lobes the capsule was pierced with a sharp needle. The needle was then worked from side to side and downward until the floor of the brain capsule was felt beneath its point. The operation was completed by thrusting the needle forward and working it around in the forebrain tissue. Although the forebrain was primarily involved, the grossness of the operation did not preclude the possibility that other parts of the brain were also affected. It will, however, be convenient in what follows to refer to fish treated in this way as "forebrainless".

Forebrainless trout recovered quickly from anesthesia when returned to cold water. Their swimming movements appeared quite normal. They differed from normal trout in that they were not as active and tended to remain for considerable periods resting quietly on the bottom. This was especially noticeable in quiet water where they would give only a perfunctory response to prodding. They oriented normally in a current and maintained their positions. Forebrainless trout did not feed actively on bits of liver dropped into the water current as normal trout did. This may have been because they lacked an olfactory sense. An operated fish would, however, snatch voraciously at food held directly in front of it on a needle. Forebrainless fish, thus fed, lived for 6 weeks or more. Eventually they died. Shortly before death there often appeared swelling and whitening of the cranium around the place where the needle had pierced it.

Damage to the dorsal part of the cerebellum was also achieved very simply. In a number of preliminary dissections of trout the relation of the cerebellum to external marks on the head was established. Under anesthesia, the cranium was pierced from the side at a point level with the dorsal part of the cerebellum. The needle was then forced gradually into the cerebellum and at the same time was worked through the tissue both dorsally and horizontally until the tip came against the wall of the cranium on the opposite side. Undoubtedly the amount of damage done varied somewhat in different individuals. However, consistent, permanent abnormalities in behavior with respect to temperature were produced as a result of the operation. In every case, except three to be mentioned later, post mortem examination of treated fish showed that the dorsal part of the cerebellum (the corpus cerebellum, Kappers *et al.*, 1936, (24), pp. 725–740) had been destroyed. It will be convenient to refer to animals thus treated as "cerebellumless".

Cerebellumless fish, after recovery from anesthesia were very active. For a day or so disturbances in equilibrium were evident and spiral swimming occurred when the fish were stimulated but these manifestations finally disappeared. The operated fish fed normally and in the running water tanks could not be distinguished from normal fish except that shadows or vibrations in the tank caused responses which were much exaggerated as compared with those of normal fish. They lived for 6 weeks or more but died eventually.

About a week before death a distinct curvature of the spine often appeared which was particularly noticeable when the animals were resting on the bottom of the tank.

These and other behavioral characteristics of the fish after ablation of the cerebellum or cerebrum have been described by Ten Cate (1935 (35)) in a review of literature on the physiology of the central nervous system of fish and by Hosch (1936–37 (23)), Wiebalck (1937 (37)), Meader (1939 (25)), Sanders (1940 (30)), and Dijkgraaf (1949 (7)) in more recent years.

In a few instances lesions in both the forebrain and the cerebellum were made in the same animal, the two operations being separated usually by several days. The operations were well tolerated by the fish.

To section the lateral line nerves a fish was lightly anesthetized and the lateral ramus of the vagus on each side was cut in two places, the first cut being made close to the operculum and the second about two millimeters posterior to this. The piece of nerve between the two cuts was removed. Trout with lateral line nerves sectioned in this way could not be distinguished from normal fish. In four instances, further interference with the lateral line sensory system was accomplished by cauterization of the lateral line organs of the head.

Results

It is necessary, in such an investigation as the present one, to decide at the outset what constitutes a "spontaneous movement". Each observer may select an apparently different criterion. One may take a change in direction as the criterion; another will choose a change in velocity; a third takes each movement of the tail fin, and so on. It is therefore important to state here that several independent observers in this laboratory, each using his own criterion of a spontaneous movement, obtained, for any given animal, curves relating temperature and frequency which differed only in the absolute number of movements recorded.

1. Normal Trout

The initial temperature at which the frequency was determined in most of these experiments was low and the temperature was raised in two or three degree steps. Averaged data of five typical experiments, showing the frequency at different temperatures, are plotted in Fig. 2*a*. It is apparent that frequency was low at low temperatures, that it rose with increasing temperature to a peak at approximately 9° C., and that it then fell, as the temperature went higher, to a minimum at 18° to 20° C. After this it rose sharply to a second peak, the "prelethal" peak and then fell abruptly as the animal died.

The usual method of raising the temperature from low to high levels was the most convenient experimental procedure and was, therefore, the routine method followed. However, the possibility existed that the observed relation between temperature and frequency was a function, in part, of the particular sequence of the temperature changes. To test this possibility, temperature

was varied randomly in four experiments. Averaged results of these are plotted in Fig. 2*b*. In this curve the prelethal peak is not apparent because it was necessary to keep the highest temperature reached in the experiments well below the lethal so that the experimental animal would not suffer damage which might affect readings at subsequent temperatures. Except for the absence of the prelethal peak the general characteristics of the relation between frequency and temperature seen in this figure were identical with those obtained when the temperature was raised regularly.

The curves in Fig. 2*a* and *b* are typical, in gross details of some 25 experiments. There was, of course, some variability in the absolute numbers of movements counted at each temperature in each experiment. Numbers of movements per 5 minutes in the first peak varied from 1397 to 732 and in the second peak from 1180 to 760. In the average curve in Fig. 2*a* the frequency of movements at the low point between the two peaks is about 35% of the frequency at the first peak. In individual experiments the frequency at this low point varied between 15 and 60% of the frequency at the first peak.

In these variations there was no apparent regularity. There were, however, consistent variations in the temperature at which the first peak occurred, this temperature being different at different times of the year. These variations may result from a general shift in temperature relations of trout. Sullivan and Fisher (1953 (34)) reported seasonal fluctuations in the temperature selected by trout in a temperature gradient, with a rise in this temperature

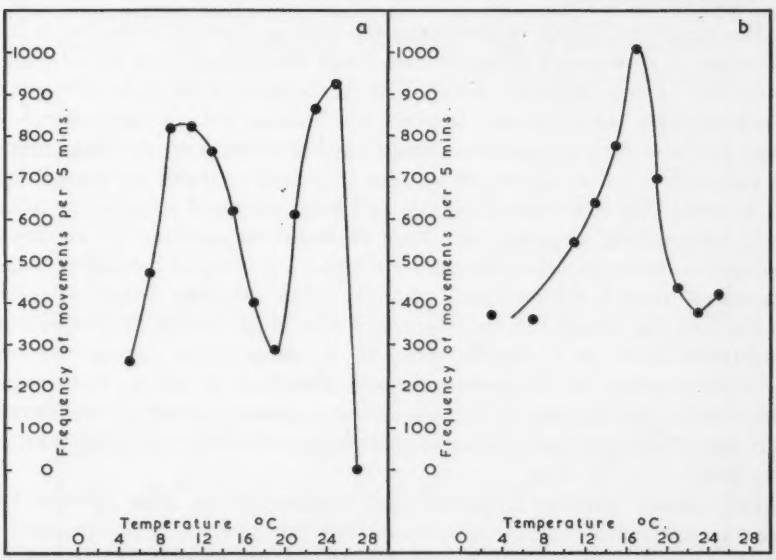

FIG. 2. Normal trout.
(*a*) Averaged data of five experiments in which the various temperatures used occurred in order from low to high.
(*b*) Averaged data of four experiments in which the various temperatures used occurred in a random order.

occurring in the spring. The five experiments averaged in Fig. 2*a*, where the first peak occurs at 9° C., were done just before this shift toward higher levels in the selected temperature took place while the four experiments averaged in Fig. 2*b*, with the first peak at 17° C., were done in midsummer, long after the spring change had been completed and when rising acclimation temperature was presumably having an effect on the temperature relations of the fish (Doudoroff, 1938 (8); Fry, 1947 (12); Graham, 1949 (14)). It is quite likely that the temperatures at which the first and second peaks in frequency of movements occur change with the seasons, and probably with acclimation temperature, just as the selected temperature does. No special inquiry into these possibilities has been made but it may be of interest to note here that occasional temperature selection experiments were done on trout from the same stock at the same time as some of the frequency experiments were being done and in these the temperature selected was always about the same as that at which the first peak in frequency of movements occurred.

It must be concluded that under the conditions of these experiments, frequency of spontaneous movements of normal trout bears a consistent relation to temperature. It is low at low temperatures, rises to a first peak at a temperature which is probably determined by season and acclimation level, decreases as temperature is raised further, then increases to a second peak which is reached at a temperature just below the lethal level.

2. Forebrainless Trout

The experiments done to determine the relation between temperature and frequency of movements in forebrainless trout were complicated by the typical inactivity of the operated animals; at all temperatures these fish moved less frequently per 5 minutes than normal animals did. Longer periods of observation at each temperature would have been required to obtain counts of the same order of magnitude as were ordinarily recorded for normal fish. If, however, the observation periods had been increased in order to record more movements, it would have been necessary to consider the chance of changes in the frequency–temperature relation occurring as a result of longer exposures to each temperature. Bullock (1955 (3)) has drawn attention to the fact that changes in the relations of rate of an activity to temperature sometimes occur very rapidly following a temperature change. It was considered better for the present purpose, therefore, to adhere to the same observation periods used in experiments on normal animals, even though this meant that the total number of movements recorded at each temperature was small.

The relation between frequency and temperature in these forebrainless fish was not quite as clear-cut as in normal fish, but, in seven of 10 experiments, more or less definite peaks occurred at temperatures between 13° and 18° C. and pronounced prelethal peaks occurred in all between 22° and 26° C. The variations in temperatures at which the peaks appeared were likely not the result of brain damage for, once again, they occurred in conjunction with

other changes which were characteristic of the regular seasonal shift in temperature relations of trout referred to above (Sullivan and Fisher, 1953 (34)).

Figure 3 presents data obtained from forebrainless fish. Results of a single experiment are given in Fig. 3*a*. Here the first peak, though greatly reduced, is still clearly suggested. In five of the 10 experiments this first peak occurred at 16° C.; combining these data, the first peak is very clearly defined (Fig. 2*b*). In three other experiments of the 10 done on forebrainless trout the relation between temperature and frequency was irregular. In one of these, only the prelethal peak appeared. In another, three peaks occurred, while in the third, the results of which are shown in Fig. 3*c*, there was no actual drop in frequency between 16° and the temperature at which the prelethal peak occurred. When results of all 10 experiments are averaged (Fig. 3*d*), the two peaks are still evident in spite of variations in temperature at which the first peak occurred and in spite of the inclusion of the irregular results.

It might be pointed out that, although numbers of movements were generally low in these experiments, the number of movements recorded at the low point between the two peaks as a percentage of the number of movements at the first peak is of the same order of magnitude as corresponding percentages already noted for normal animals, being 54, 15, and 62% for parts *a*, *b*, and *d* respectively of Fig. 3.

It seems necessary to conclude from these data that the essential characteristics of the relation between frequency and temperature in forebrainless trout did not differ significantly from those seen in normal animals. The

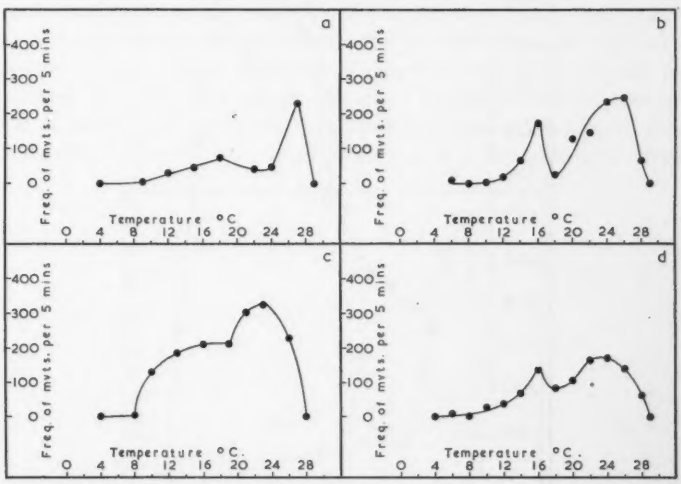

FIG. 3. Forebrainless trout.
(*a*) Results of a single experiment.
(*b*) Averaged data of five experiments with the first peak at 16° C.
(*c*) Results of a single experiment.
(*d*) Averaged data of all experiments done.

frequency of movements made by these fish increased with temperature to a peak at an intermediate temperature, then decreased as the temperature rose, and finally increased to its highest value at a temperature just below lethal level.

3. Cerebellumless Trout

In a general way the absolute number of spontaneous movements made by cerebellumless fish was comparable to the number made by normal animals, but the relation between temperature and the frequency of movements made by cerebellumless trout was quite different. The averaged observations of nine strictly comparable experiments involving eight fish appear in Fig. 4. These data are representative in every way of those obtained in the individual experiments. Three independent observers produced similar curves, differing only in absolute number of movements counted. The same result was obtained regardless of whether the experiment was done 1 day or 3 weeks after the operation. Repeated experiments on the same individual did not produce any change in the result—the relation between frequency of movements and temperature was the same in the last test as in the first. The same relation was seen when the temperature was progressively raised as when it was varied at random.

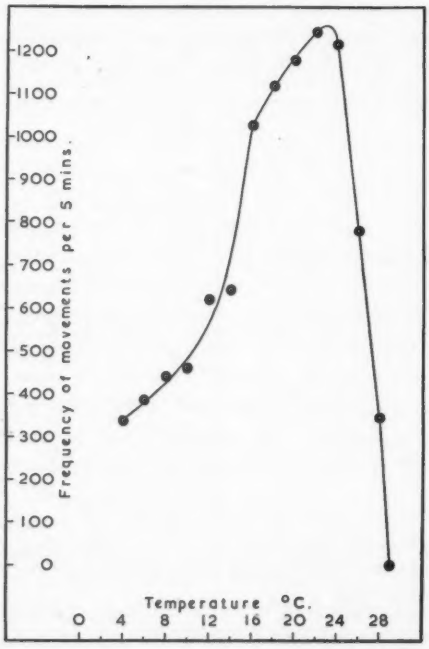

FIG. 4. Cerebellumless trout. Averaged data of nine experiments.

Figure 4 shows that frequency of movements in cerebellumless trout rose more or less regularly to a peak which occurred somewhat below the lethal temperature and then fell off as the animals died. Thus the first peak, which appeared at 15° C. in the frequency response of normal animals tested at the same time (October) was not evident in the frequency response of cerebellumless trout.

It is of interest that in one operation damage was done unintentionally to the ventral instead of to the *dorsal* part of the cerebellum. In this animal, as well as in several in which the ventral part of the cerebellum was deliberately involved, the relation between frequency of movements and temperature was the same as in normal animals.

It is apparent that damage to the dorsal part of the cerebellum disturbs the frequency response to temperature, obscuring or eliminating the first peak in frequency, which normally appears at an intermediate temperature.

4. Forebrainless Cerebellumless Trout

Observations of the relation of frequency of movements and temperature were made on four trout in which both forebrain and cerebellum were damaged. The relation between frequency and temperature in each showed a distinct peak at 13°–18° C. In Figure 5 the results given by the four forebrainless, cerebellumless fish are averaged. From the figure it can be seen that the relation between frequency and temperature was very similar to that of normal fish, except that absolute numbers of movements are somewhat less. It appears that the first peak, which is characteristic of normal and forebrainless fish, but absent in cerebellumless fish is once more a definite feature of the temperature–frequency relation in forebrainless, cerebellumless fish.

Consideration of the observations on normal, cerebellumless, and forebrainless trout (omitting results for forebrainless-cerebellumless) would lead to the conclusion that the relation of frequency of movements to temperature under the experimental conditions employed here was a function of the cerebellum. Since, however, an apparently normal temperature frequency

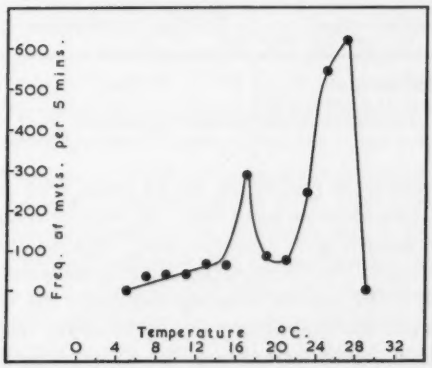

FIG. 5. Forebrainless, cerebellumless trout. Averaged data of four experiments.

relation exists in experimental fish if cerebellum damage is accompanied by forebrain destruction, it is clear that the pattern of behavior here under study must result from interaction of parts of the central nervous system. Much more work will be needed before the mechanism which sets the pattern of frequency vs. temperature can be understood.

5. Lateral Lineless Trout

Rubin (1934–35 (29)) has published observations which suggest that receptors in the trunk lateral line system might be concerned with the effect of temperature on the frequency of movements. He observed that fish which normally became active at a specific response temperature, in an environment where temperature was rising rapidly, failed to give this response after section of the trunk lateral line nerves. Furthermore, Hoagland (1932–33 (21)) has recorded temperature-sensitive, spontaneous impulses in the lateral line nerves of the trunk of a number of fish and, in goldfish, has traced these impulses into the cerebellum (Hoagland, 1934–35 (22)). These observations of Rubin and Hoagland, along with those described above, which showed that the normal frequency response disappeared when part of the cerebellum was damaged, suggested an association of the lateral line of the trunk with the frequency response to temperature.

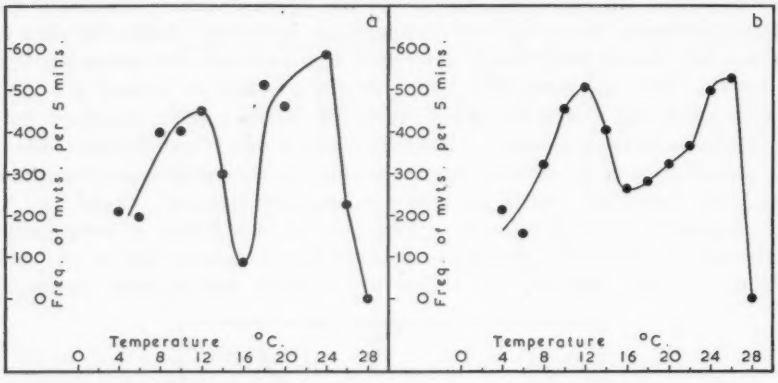

FIG. 6. Lateral lineless trout.
(*a*) Averaged data of 11 experiments.
(*b*) Averaged data of the six experiments which individually could not be distinguished from controls.

Fifteen experiments were performed on 13 trout with lateral line nerves sectioned near the operculum on each side. In four of these the head lateral line organs were also destroyed by cauterization. In four of the 15 experiments the fish died at unusually low temperatures and these were therefore omitted from consideration. The curves relating frequency to temperature in six of the 11 experiments left (involving five fish) could not be distinguished from curves obtained from normal animals, two distinct peaks appearing in each experiment. Results of these six experiments are averaged in Fig. 6*b*.

The data of the five remaining experiments (involving four fish) did not, when taken individually, provide unequivocal evidence for two peaks. Irregularities were not consistent, however, and when these data were combined the average result did show the two peaks (Fig. 6*a*).

The conclusion must be drawn that the frequency of spontaneous movements of lateral lineless trout varies with temperature in much the same way as it does in normal trout. It appears that the lateral line sensory system either is not involved in the responses studied here or, if normally involved, can be readily replaced by some other, as yet unknown, mechanism.

Discussion

Cases have already been cited in which the relation observed between activity and fixed levels of humidity appeared to account wholly or in part for aggregation in or selection of a particular part of an environment which was non-uniform with respect to humidity. Thus, the fact that the activity of cockroaches is less in a dry environment than in a relatively "wet" one may appear to account for the fact that in a gradient of humidity these organisms select the condition in which they are least active. Arguing similarly, it would appear from the present investigation that in a temperature gradient trout should be found at temperatures below 5° C., and between 15° and 21° C. (depending on season), for it is at these temperatures that the trout were least active. Actually, however, as has been shown elsewhere (Fisher and Elson, 1950 (10); Sullivan and Fisher, 1953 (34)), and confirmed here, trout select temperatures between 7° and 17° C. (depending on season), and at the selected temperature, as the present investigation shows, activity is *high* not low. Other cases in which it has similarly been shown that the temperature selected in a gradient is the temperature of greatest activity of the animals when held in various constant temperatures have been reported by Nicholson, 1934 (27); Gunn and Hopf, 1941–42 (18); Gunn and Walshe, 1942 (20).

There is, of course, no *a priori* justification for assuming that behavior of organisms in a gradient of some factor such as temperature will be directly deducible from their behavior at each of several uniform levels of that factor. The comparisons have been made, however, but the apparently logical prediction that the organisms will select, in the gradient, that level at which they are least active under uniform conditions has as often been unsubstantiated or actually controverted by direct observation, as it has been confirmed.

If there are a few cases in which the responses under constant conditions obviously do not explain, in whole or in part, the behavior in gradient conditions, then it seems necessary to exercise great caution in correlating observations under the two different sets of circumstances even where they do appear to support each other, since it is possible that the agreement is of a coincidental nature and not owing to a cause and effect relation. Further work in all these cases is clearly needed before sound conclusions about such cause and effect relations can be finally formulated.

The fact that the temperature at which the first peak in frequency of movements of trout occurs is the temperature selected by comparable trout in a gradient may indicate that these two activities are not directly related, but it is of interest in connection with the general temperature relations of the animals. It seems quite likely that this particular temperature may have some special significance for the fish. It is the same temperature at which the distance moved by trout in response to an electric shock is maximum (Elson, 1942 (9); Fisher and Elson, 1950 (10)) and probably the same temperature at which the maximum cruising speed that trout can be forced to maintain is observed (Sullivan, 1954 (33)), but see also Fry and Hart, 1948 (13)). The possibility that these several activities are causally related is under investigation at the present time.

Acknowledgments

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STUDIES ON STRONGYLOIDES OF PRIMATES

I. MORPHOLOGY AND LIFE HISTORY OF STRONGYLOIDES FÜLLEBORNI VON LINSTOW, 1905[1]

PREMVATI[2]

Abstract

A detailed description is given of the parasitic females and free-living stages of *Strongyloides fülleborni* von Linstow, 1905, from Indian rhesus monkeys.

Introduction

The genus *Strongyloides* from the intestine of man has long been known. In 1905 von Linstow (10) described the first species, *S. fülleborni* of lower primates, from material collected by Fülleborn from the intestines of *Anthropopithecus troglodytes* and *Cynocephalus babuin* from Africa. This species has since been recorded from *Simia sinicus* L. by Gonder (6); from *Macacus cynomolgus*, *M. rhesus*, and *M. nemestrinus* by Weinberg and Romanovitch (13); from *Pithecus rhesus* and *Macacus* sp. by Sandground (12); from *Papio papio* by Goodey (7); and from *Cercopithecus pygerethrus*, *Papio porcarius*, and man by Blackie (2). Two additional species have since been described from lower primates, namely, *S. cebus* by Darling (5) from *Cebus hypoleucus* from the Panama Canal Zone, and *S. simiae* by Hüng and Hoeppli (8) from *Macaca* sp.

The author's examination of the intestines of over 1500 rhesus monkeys from India showed a heavy infection of *Strongyloides* which in morphology, life history, and biology resemble *S. fülleborni* in all respects and thus are described as such. In 1926 Goodey (7) redescribed this species but its detailed life history was still unknown.

Materials and Methods

A continuous supply of entrails of rhesus monkeys from the Institut de Microbiologie, Université de Montréal, facilitated this work. In order to avoid contamination with free-living nematodes (as may happen when faeces are used) only the contents of the small intestines were used in the preparation of cultures. The contents of 30 to 40 intestines at a time were cultured in cow faeces (sterilized at 250° F. under 17 lb. pressure to ensure the death of all helminth eggs) mixed with cocoanut charcoal (80 mesh). The cultures were placed in Petri dishes the lids of which were lined with filter paper which was moistened daily to maintain the correct humidity. For normal growth the cultures were kept just moist and incubated at 25° C., the optimum temperature.

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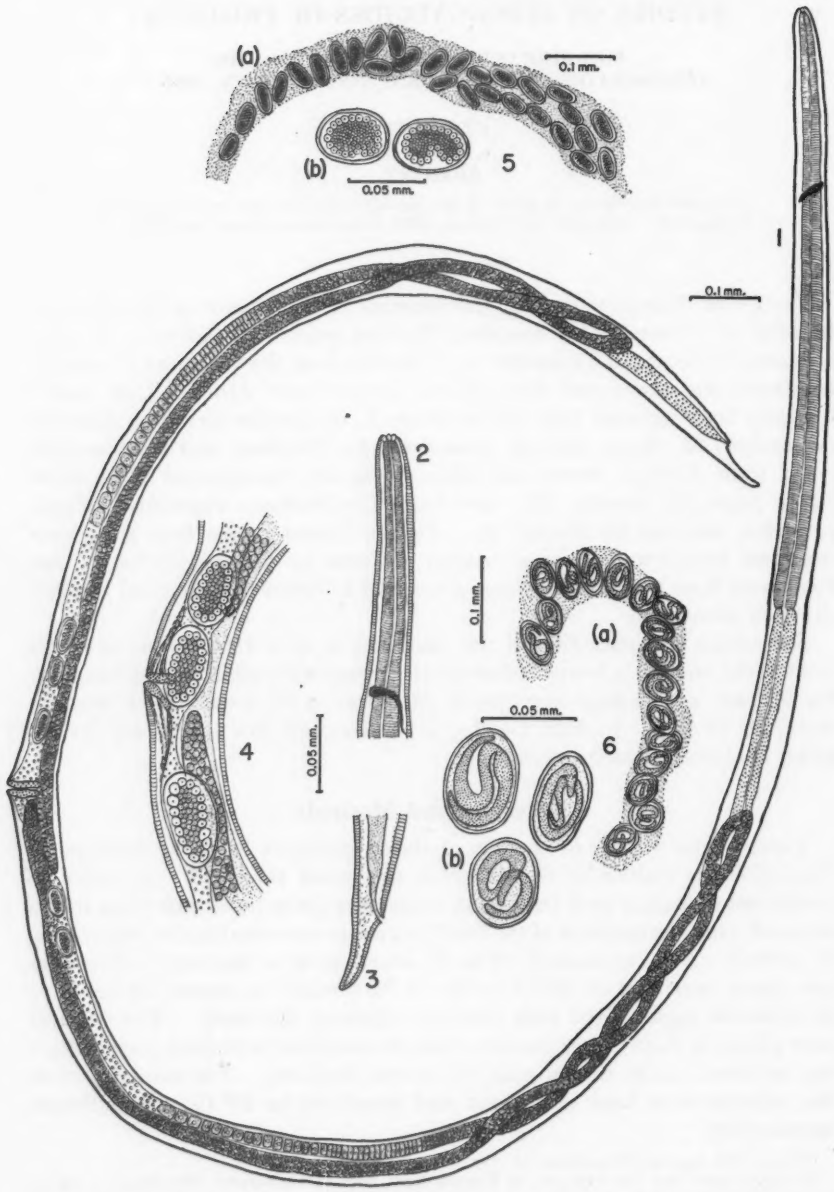
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

[2]Margaret McWilliam Fellow (awarded by Canadian Federation of University Women). At present on leave from the Department of Zoology, University of Lucknow, Lucknow, India.



FIGS. 1–6. Parasitic phase of *Strongyloides fülleborni*. FIG. 1. The adult parasitic female. FIG. 2. Anterior end. FIG. 3. Tail end. FIG. 4. Vulvar region, lateral view. FIG. 5*a*. String of eggs in their early development. FIG. 5*b*. Eggs under high magnification. FIG. 6*a*. String of eggs fully embryonated. FIG. 6*b*. Eggs under high magnification.

Parasitic females were picked out from the intestinal contents after the mucus had been dissolved in a saturated solution of sodium bicarbonate. In order to obtain free-living worms at all stages of their development, the cultures were baermanned at hourly intervals for a period of 4 days from the commencement of incubation.

The material was studied alive, fixed in hot 70% alcohol or 5% formal saline, and cleared with glycerine and lactophenol. Living specimens were examined in 1% Lugol's iodine, neutral red, methylene blue, and methyl green intra-vitam stains. A new technique was developed for studying the larvae alive. The worms were left in a solution of 100 cc. of 1% brilliant cresyl blue, 2 g. gum arabic, and 1 cc. commercial formalin for 20 to 25 minutes, and then examined under the microscope. This method, which has a narcotic effect on the larvae, facilitated examination of cuticle striations, the nervous system, and excretory glands. Sublethal doses of ultraviolet radiation also revealed certain morphological structures not otherwise seen.

Morphology of *Strongyloides fülleborni*

The Parasitic Female

The parthenogenetic female (Fig. 1) is filiform in shape, whitish with a slight yellow tinge in color, and lies between the villi of the small intestine with its anterior end reaching to the muscularis mucosa. The cuticle is thin and (using the cresyl blue – gum arabic technique) the striations are clearly seen throughout the body. The anterior end tapers slightly. The centrally placed mouth (Fig. 2) is surrounded by six papillae and leads directly to the oesophagus. There is no distinct buccal cavity.

Total length is 3.6 to 4.6 mm. and maximum breadth at the region of the vulva 0.050 to 0.068 mm. The oesophagus is 0.75 to 0.95 mm. long, and varies from one-fourth to one-fifth of the total body length. The oesophagus is somewhat narrower at the anterior end and becomes broader at its base but there is no bulb or demarcation; it is about 0.016 mm. wide at its anterior end and about 0.030 mm. wide at its base. The intestine continues from the oesophagus straight to the short rectum (making a base for the coils of the ovary). The tail (Fig. 3) has a fingertip-like blunt end and measures 0.050 to 0.075 mm. in length.

The nervous system is represented by a band around the oesophagus at a distance of 0.19 to 0.25 mm. from the anterior end.

The reproductive system consists of an anterior and a posterior ovary both of which arise slightly anterior to the vulva as blind tubular sacs. The ovary is divided into an anterior germinal zone, where rapid division of relatively small cells takes place, and a growth zone, where gradual increase in size of the oögonia takes place. It extends anteriorly or posteriorly and is then reflexed, forming coils with the intestine as its base. The vulva lies in the posterior half of the body usually just anterior of the last third. The distance from the anterior end to the vulva varies between 2.30 and 2.86 mm., and from the vulva to the anus between 1.21 and 1.63 mm. Both ovaries are reflexed

but the coils show great variation. In some, the anterior ovary has as many as six coils around the intestine while the posterior has only one. In others, both ovaries show an equal number of coils. In general, however, the anterior ovary has four and the posterior, two coils. The distance between the base of the oesophagus and the reflexed portion of the anterior ovary, or between the anus and the reflexed portion of the posterior ovary, depends upon the age of the parasite. The two uteri meet in the region of the vulva (Fig. 4); there is no ovejector. The vulva communicates with the uterus through a vagina which occupies about one-third of the total body width. The vulva is a mid-ventral transverse slit with two slightly bulging lips. Receptacula seminalis are absent.

The uterus never contains more than 8 to 10 eggs, the eggs nearest the vulva being the most developed, but inside the uterus development does not proceed beyond the morula stage. Although never more than 10 eggs were seen in the uterus at a time, strings of eggs seen in the intestinal contents showed as many as 30 eggs (Figs. 5 and 6). The eggs of some strings were still in the morula or the tadpole stage while other strings had fully embryonated eggs. In each string, however, the eggs were always at the same stage of development suggesting that they had been laid at the same time.

The eggs are small, 0.050 to 0.063 mm. long and 0.026 to 0.035 mm. wide, and oval in shape.

No parasitic male was seen in any of the some 1500 intestines examined.

The parasitic female of *S. fülleborni* as described by von Linstow (10) has a smooth cuticle and a length of 3.78 mm. Chandler (4), Sandground (12), and Goodey (7) have shown that the cuticle is finely striated, the striations being visible only under higher magnifications. The writer agrees with these authors but finds that the cuticular striations can be seen, by using the new technique described above, under the lower power of the microscope. In writing of the length of the parasitic female Goodey (7) wrote:

> "The lengths of the specimens examined by the writer, all sexually mature forms, agree remarkably closely with Sandground's figures, as they form a gradual series from 2.03 to 2.96 mm. and in view of this it seems possible that von Linstow set down 3.78 mm. in error for 2.78 mm."

The length of the parasitic females examined by the writer varied from 3.62 to 4.6 mm. The identity of these forms as *S. fülleborni* is undoubtedly correct in that in the free-living generation the adults completely resemble those described by von Linstow, Sandground, and Goodey. In the author's opinion, therefore, von Linstow was correct in giving the length of the female as 3.78 mm.

Von Linstow gave the number of eggs present in the parasitic female as about 30. Sandground and Goodey did not discuss the number but from the diagram given by Goodey, it would appear that he too found very few eggs in the uterus of the female. The writer has examined more than 500 adult parasitic females and in all the number of eggs was under 10.

The Life History of *Strongyloides fülleborni*

The genus *Strongyloides* in the free-living stage shows two types of life cycle. The direct or homogonic type is that in which the eggs of the parasitic female hatch into rhabditiform larvae which metamorphose directly into infective or filariform larvae. The indirect or heterogonic type is that in which the eggs of the parasitic female give rise to rhabditiform larvae which metamorphose into free-living adults; these in turn, after fertilization, lay eggs from which rhabditiform larvae hatch which, in turn, develop into infective larvae.

Under optimum conditions development of the species in apes and monkeys is always indirect or heterogonic, showing thereby an alternation of generations.

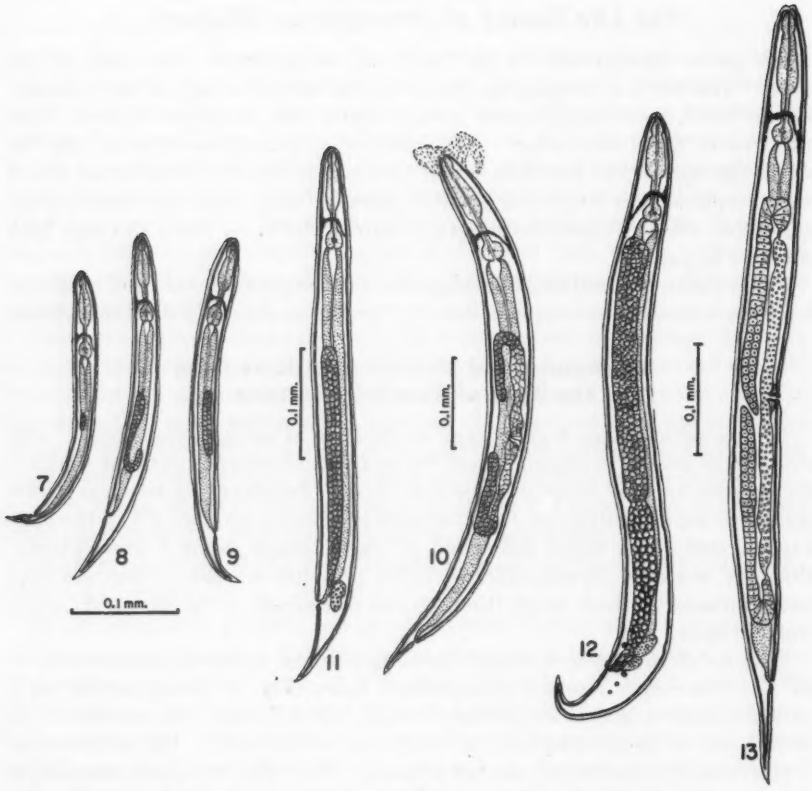
Development of Free-living Adults from the Eggs of Parasitic Females

The parasitic female lays its eggs in the form of strings (Figs. 5, 6). The author was unable to examine fresh faeces from monkeys to find out whether the eggs are hatched inside or outside the host. *In vitro* experiments in which eggs were kept in culture at 37° C. showed that not more than 5% of the eggs hatched and those which did failed to show normal indirect development. Previous work on *Strongyloides* of lower primates has shown that the eggs hatch outside the host in all three species concerned, *S. fülleborni*, *S. cebus*, and *S. simiae*.

Eggs hatch after 6 to 8 hours' incubation at the optimum temperature of 25° C. The newly hatched rhabditiform larva (Fig. 7) is surrounded by a cuticular sheath and is an actively feeding motile stage. It measures 0.23 to 0.28 mm. in length and 0.015 to 0.018 mm. in thickness. The anterior end is tapered and rounded and the tail pointed. The fully developed oesophagus shows a characteristic rhabditiform shape and measures 0.065 to 0.078 mm. in length. The nerve ring is present around the middle of the isthmus. The oesophagus joins the straight intestine, which terminates in a short rectum opening at the anus, a distance of 0.028 to 0.046 mm. from the tail end. The genital primordium measures 0.018 to 0.023 mm. and is represented by a small group of cells in mid-body attached to the intestine, 0.117 to 0.137 mm. from the anterior end.

After the first molt, which takes place between 4 and 6 hours after hatching, male and female larvae can be distinguished. The size is the same in both sexes but in the female (Fig. 8) the gonad becomes elongated and begins to be reflexed; in the male (Fig. 9) it is straight and stouter.

A phase of growth follows this molt. Basir (1) noted four molts in *S. papillosus*, the first between 7 and 10 hours, the second between 14 and 16, the third at about 21, and the fourth at 28. The second molt in *S. fülleborni* was observed by the author after 20 hours. At this stage the cuticle is seen to break at the anterior end, but before the old cuticle is cast the new thin cuticle is visible surrounding the larvae. In female larvae (Fig. 10) the gonads have reflexed on both sides and the vulva has made its appearance although there



FIGS. 7–13. Development of free-living adults from eggs from parthenogenetic female. FIG. 7. Rhabditiform larva, just hatched. FIG. 8. Female larva after first molt. FIG. 9. Male larva after first molt. FIG. 10. Female larva after second molt. FIG. 11. Male larva after second molt. FIG. 12. Male larva after third molt. FIG. 13. Female larva after third molt.

is as yet no aperture. In the male larvae (Fig. 11) the genital organ is straight but has not attained its full size; the posterior end is slightly broader than that of the female larvae and there are slight traces of the spicules.

After the third molt, which takes place after 28 to 30 hours, the larvae become immature adults. The male (Fig. 12) is smaller than the female and measures about 0.6 mm. in length and 0.04 to 0.05 mm. in width. It has the curved tail characteristic of the adult male. A pair of spicules, five pairs of anal papillae, and the rectal glands can be seen clearly. The testis, vas deferens, and ejaculatory duct are well developed, and the gubernaculum shortly makes its appearance.

The immature female (Fig. 13) has an elongated straight body and measures 0.7 to 0.8 mm. in length and 0.03 to 0.04 mm. in width. Each ovary is completely formed, the receptacula seminalis is visible, and the uteri run towards

the middle of the body to join a very short vagina which opens into the fully formed vulva. The vulvular lips are not as prominent as in adults and show no bulging. The body is of uniform width in the middle region and the very characteristic curve just below the vulva of the mature female is absent.

Free-living Adults

Female

The females, always more numerous than the males in cultures, were collected in thousands after baermanning of a culture maintained for 48 hours at 25° C.

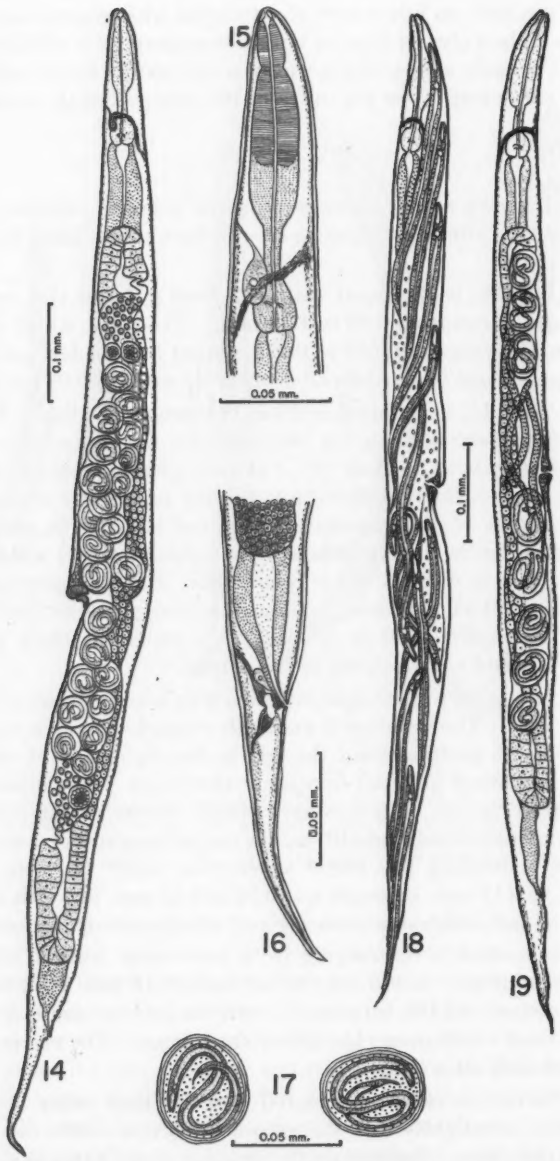
The body (Fig. 14) is elongated with a tapered anterior end and pointed tail. The length varies from 0.90 to 1.19 mm. The body, which is broadest just anterior to the vulva, is 0.059 to 0.076 mm. at its broadest point and becomes more narrow just below the vulva where its width is 0.046 to 0.052 mm.

The mouth (Fig. 15) is terminal and has two prominent lips. There is no prominent constrictuon forming an expansion in the shape of a crown as described for *S. papillosus* by Basir (1). The oesophagus measures from 0.143 to 0.156 mm. and is of the rhabditiform type with an anterior corpus followed by a narrow isthmus which is succeeded by an end bulb. The pseudobulb of free-living nematodes is missing although the corpus shows a widening at its base. The corpus is divided by a constriction into an anterior portion, measuring 0.02 to 0.03 mm. and a more muscular posterior portion. The spears described by Kreis (9) in *S. simiae* are really the thick pharyngeal muscles which extend to two-thirds of the corpus.

The oesophagus leads to a straight intestine with a well developed dilatation at its anterior end. The intestine is markedly constricted in the region of the vulva, changing its position from the left to the right side of the body, a characteristic feature of all adult females of this genus. The intestine in the posterior region (Fig. 16) ends in a very small, slender rectum, the muscles of which are not well developed although a pair of long muscles and a pair of short muscles controlling the rectal valves can easily be seen. The tail measures 0.11 to 0.13 mm. in length and 0.02 to 0.03 mm. in width at the level of the anus, and has a slight curve on the anal side becoming pointed at its tip.

The nervous system is represented by a nerve ring lying in the isthmus region of the oesophagus just above the end bulb, 0.15 mm. from the anterior end. The excretory cell lies between the isthmus and the end bulb and leads to a very fine duct which opens just below the corpus. The excretory pore is very small and difficult to see.

The two ovaries are reflexed, situated opposite each other in mid-body. Their blind ends are slightly club-shaped and their tips overlie one another in the region of the vulva. Sometimes the anterior ovary extends slightly over the posterior; at other times, the posterior ovary is extended to the anterior of the vulva. The anterior ovary extends anteriorly and is reflexed below the anterior dilatation of the intestine. The distance from the base of the oesophagus to the reflexed portion of the ovary varies according to its age.



FIGS. 14–19. Free-living adult females. FIG. 14. Fully mature female with embryonated eggs in uterus. FIG. 15. Anterior end. FIG. 16. Tail end. FIG. 17. Embryonated eggs under high magnification. FIG. 18. Old dying female with rhabditiform larvae hatched inside body. FIG. 19. Adult female still alive and active, with rhabditiform larvae hatched inside the uterus.

Similarly, the posterior ovary extends posteriorly and is reflexed in front of the rectum, the distance also being variable with age. Before opening into the uterus each ovary has a very prominent seminal receptacle which is somewhat elongated, much wider than the ovary, and very clearly seen when full of spermatozoa after 36 hours of incubation and before the fully embryonated eggs obscure their presence. The seminal receptacles lead into the uterus, the walls of which are extremely distended when full of embryonated eggs.

Each ovary is a tubular sac in which germinal cells multiply rapidly in its anterior, small, germinal zone, and develop in size to oögonia in its long, growth zone. As they mature, the oögonia pass down the tube and are fertilized in the seminal receptacle. The shell is formed in the proximal portion of the uterus.

The vulva is situated almost in mid-body, 0.52 to 0.63 mm. from the anterior end and 0.40 to 0.49 mm. from the anus. Its appearance is characteristic in the mature female, with a prominent enlarged anterior lip overhanging the aperture, and a small posterior lip. There is a marked constriction in the body width just below the vulva giving it a waist-like appearance. The bases of the vulva lips are connected with the body and the uterine walls by means of radial muscles which control the opening. The prominence of the lips is so pronounced that there is almost no vagina.

The eggs (Fig. 17) when laid are fully embryonated and measure 0.049 to 0.064 mm. by 0.026 to 0.033 mm. The number of eggs in the uterus varies from 15 to 30. Each egg is surrounded by three layers.

The female begins to lay fully-embryonated eggs after 52 hours of incubation at 25° C. These eggs take a very short time to hatch and sometimes all the eggs are embryonated in the uterus before being laid. When the female is examined continuously under the binocular microscope it is seen that, generally, practically a whole set of eggs is laid at once, only a few in an early stage of development remaining in the uterus. The females then again copulate, store spermatozoa in the seminal receptacle, and pass fertilized oögonia to the uterus. The egg-laying process is thus repeated a number of times.

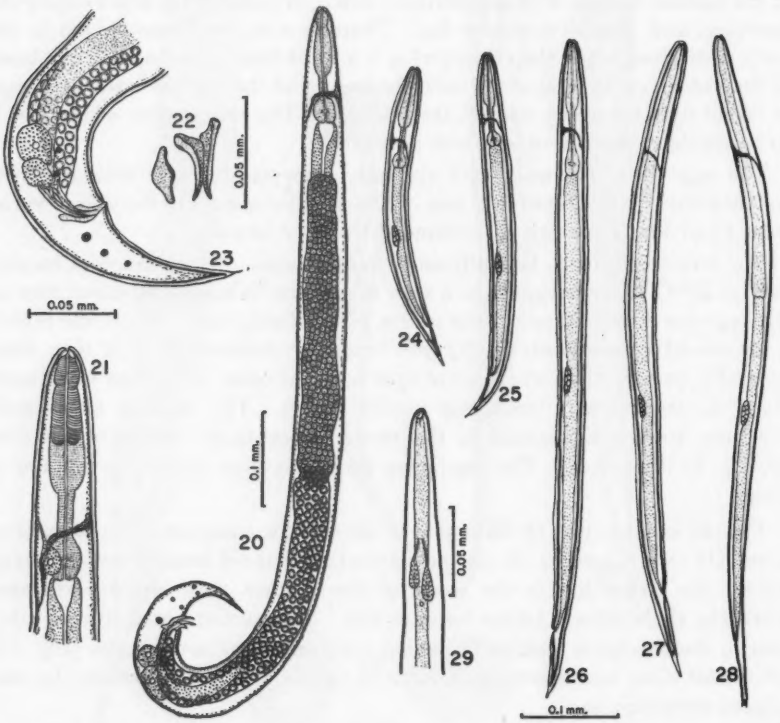
The old females (Fig. 18) were seen to have motile embryos inside the uterus. Looss (11) and Cameron (3) also noted the fact that old females become viviparous; the larvae live in the tissue of the mother until she disintegrates, when the rhabditiform larvae become free. The author noted that in addition to the old dying females becoming viviparous, mature females (Fig. 19), while still alive and active, give birth to larvae and can, therefore, be considered ovoviviparous.

While the mature females show the very characteristic vulvar region with prominent lips and waist-like appearance after 48 hours' incubation, this character is gradually lost as they become older. In old, dying females where all larvae hatch within the host, the body is of uniform width and the waist-like appearance totally disappears.

Male

In normal cultures, free-living males appear simultaneously with the females after 48 hours' incubation, but the males may also appear under unfavorable conditions which would cause the females to change from rhabditiform to filariform larvae, or even prevent any development at all.

The male (Fig. 20) is smaller than the female, always has a curved pointed posterior end, and measures 0.80 to 0.95 mm. in length and 0.038 to 0.046 mm. in width. The anterior end (Fig. 21) is tapered and rounded and is similar to that of the female except that the oesophagus is smaller, measuring 0.11 to 0.13 mm. in length. The intestine opens at the posterior end into a cloaca, which is situated 0.08 to 0.10 mm. from the posterior tip. The nerve ring, excretory pore, and excretory cell are as in the female and occupy the same position, opening in the isthmus region below the corpus. Two rectal glands are visible at the posterior end.



FIGS. 20–23. Free-living adult males. FIG. 20. Fully mature male. FIG. 21. Anterior end. FIG. 22. Spicules and gubernaculum. FIG. 23. Posterior end.

FIGS. 24–29. Development of infective larvae from the eggs of free-living adults. FIG. 24. Rhabditiform larva, just hatched. FIG. 25. Rhabditiform larva at time of first molt. FIG. 26. Rhabditiform larva before the second molt. FIG. 27. Preinfective larva after the second molt. FIG. 28. Infective larva after third molt. FIG. 29. Anterior end of infective larva showing excretory pore and a pair of glands.

The reproductive system consists of a single, long, stout, straight cylindrical testis which extends to the base of the dilatation of the intestine in mature specimens. At no stage of development did the testis show any reflexed portion such as mentioned by Goodey (7) for *S. fülleborni* and by Kreis (9) for *S. simiae*. It continues into an elongated vas deferens which is full of spermatocytes and practically of the same width as the testis; a marked constriction separates it, in the form of a seminal vesicle, from the testis. The vas deferens is followed by a small ejaculatory duct which opens, in common with the intestine, into the cloaca. There are two spicules (Fig. 22), equal in size and similar in shape; they measure 0.33 to 0.39 mm. in length, have a knob-like proximal end, and a pointed distal end. The gubernaculum, 0.022 to 0.26 mm. in length, with a characteristic shape, overlies the spicules. It is flattened and broad in the middle with finger-like tips at its two ends. Both the spicules and gubernaculum are provided with retractor and protractor muscles.

There are five pairs of anal papillae (Fig. 23), two preanal and three postanal. To date, only two pairs of anal papillae—one preanal and one postanal—have been described from *S. fülleborni*, *S. cebus*, and *S. simiae*. Basir (1) described four pairs—two preanal and two postanal—but mentions the probability of more being present which could not be seen owing to their small size. In the author's specimens, two pairs—one preanal and one postanal—are very distinct, while the other three pairs are small and could be seen only under the highest magnification.

Development of Infective Larvae from the Eggs of Free-living Adults

The rhabditiform larvae (Fig. 24), hatched from the fertilized eggs of free-living females, are similar in shape and size to those developed from eggs of the parthenogenetic female. The larvae grow to a length of 0.3 to 0.4 mm. when the first molt takes place (Fig. 25). This molt is followed by an active growth phase and the larva (Fig. 26) becomes elongated. While still rhabditiform the oesophagus also becomes somewhat elongated. The genital primordium can be seen and the larva is still enclosed in a cuticular sheath. The second molt then takes place. The oesophagus (Fig. 27) becomes much longer, loses its characteristic rhabditiform shape, and although slightly narrower at its anterior end, becomes of uniform width. The other outstanding change which follows this molt is the formation of a trifid tail. This larva, which may be called the preinfective larva, is still enclosed in its cuticular sheath which is cast on the third molt and the infective larva (Fig. 28) emerges, naked, actively moving, but non-feeding.

The time taken for these molts depends upon environmental conditions. If these are unfavorable, most of the first-stage rhabditiform larvae die but if they reach the second molt stage, 90% survive and change quickly to preinfective stages.

Only the first-stage rhabditiform larvae require semisolid, well-aerated conditions; the other stages are able to develop even in water and without food.

Whether developed from eggs of the parasitic female or the free-living female, first-stage rhabditiform larvae are similar in their morphology. The larvae of free-living females are, however, much more resistant to adverse conditions than those from the parasitic female.

Infective Larvae

The sheathless, non-feeding infective larva (Fig. 28) has a cylindrical body with a tapering, rounded anterior end and a slender posterior end. Their size varies slightly with the culture media in which they develop. They measure from 0.47 to 0.75 mm. in length and 0.013 to 0.018 mm. in width. The head is rounded and has six very small knob-like papillae. There is no buccal cavity, the mouth passing into the oesophagus directly. The elongated oesophagus is from 0.20 to 0.30 mm. long; it is narrowest at its anterior end and broadest at its base where it is continued into the intestine of the same width. The intestine tapers gradually at its terminal end and is followed by a small rectum. The anus is situated about 0.05 mm. from the tail end. The tail is slender, varies in length from 0.046 to 0.052 mm., and has a characteristic trifid condition at its terminal end.

The nervous system consists of a nerve ring encircling the oesophagus 0.08 to 0.15 mm. from the anterior end. The germinal primordium, about 0.015 to 0.02 mm. in length, is oval in shape, situated on the side of the intestine 0.28 to 0.40 mm. from the anterior end.

While working with the effect of radiation on these larvae, we observed some important morphological structures. With sublethal doses of ultraviolet radiation, the excretory pore is clearly seen at a distance of 0.10 to 0.18 mm. from the anterior end of the body (Fig. 29). Just above the excretory pore a pair of glands with their separate ducts running anteriorly for some distance and then opening into the oesophagus is seen. These glands probably are helpful to the infective larvae during penetration. The right gland is slightly longer than the left, but each has an oval shape with a distinct nucleus. If, after ultraviolet radiation, these larvae are treated with neutral red intra-vitam stain, these glands are seen more clearly. It would be impossible to observe them in live specimens.

Summary

No males occur in the parasitic generation of *S. fülleborni*. While never more than 8 to 10 eggs were observed in the uterus of the parasitic female, strings of as many as 30 eggs, at the same stage of development, were found in the intestinal contents. The eggs are not embryonated inside the uterus of the parasitic female but are laid at an early stage. In the case of *S. fülleborni*, under optimum conditions, development is always indirect or heterogonic. There is only one generation of free-living adults. Eggs of the parasitic female hatch into rhabditiform larvae which molt three times before becoming

mature, free-living adults. Three molts also occur in the development of infective larvae from the rhabditiform larvae hatched from the eggs of the free-living generation. A pair of glands which perhaps help in penetration were seen for the first time in the infective larvae. The free-living males have five pairs of anal papillae, two preanal and three postanal.

Acknowledgments

The author acknowledges with gratitude the co-operation of the Institut de Microbiologie, Université de Montréal, and the Laboratory of Hygiene, Department of National Health and Welfare, in making available over a period of time the viscera of more than 1500 rhesus monkeys. Without this cooperation the present study would have been impossible. She is indebted to Professor T. W. M. Cameron for his constant help and valuable criticism.

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IDENTIFYING RECENT AND FOSSIL BARNACLES[1]

IRA E. CORNWALL[2]

Abstract

The study of the microstructure of the shells of sessile barnacles shows that in many of them there is a figure, or pattern, that is constant in both recent and fossil shells of the same species and this can be used in identification.

It is quite easy to find the pattern in the shell and the method of doing it is fully described in this paper.

Introduction

To determine the species of a barnacle is often a very difficult matter, there are so few that can be recognized by their external appearance. Usually the only sure way of identifying a species is to dissect the animal, examine the mouth parts, cirri, and external and internal sculpture of the cover plates and shell, then compare these with the illustrations of the many well-known species. To determine the species of a fossil barnacle is even more difficult. Of course the body is gone, very often the cover plates are missing, and the whole shell may be embedded in such hard matrix that the internal sculpture and structure of the shell cannot be examined. But in the shells of many sessile barnacles there is another means of identification. There is a little pattern in the shell that is the same in both recent and fossil shells of the same species. This pattern lies between the inner and outer lamina of the shell and can be seen by cutting and polishing the cut surface as described in this paper. Even a small fragment of a sessile barnacle shell can often be determined in this way.

Material

The material for this study was obtained from the following sources: The United States National Museum, Washington, D.C.; British Museum, London, Eng.; Provincial Museum, Victoria, B.C.; The Australian Museum, Sydney, Australia; Canadian National Museum, Ottawa, Canada; Woods Hole Oceanographical Institution, Woods Hole, Mass.; The University of Southern California and The Allan Hancock Foundation, Los Angeles, California; Rhodes University, Grahamstown, South Africa; Biological Station, St. Andrews, N.B.; The Pacific Biological Station, Nanaimo, B.C.; General Biological Supply House, Chicago, Ill.; personal and numerous private collections.

Method of Preparing Shell

The patterns in the shells of sessile barnacles were first seen by Alessandri (1) when he made thin sections of the shell. This is a good method but as it takes much time to make a thin section, many experiments were made to find an easier way to see the patterns. It was eventually found that if the

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shell was cut at about right-angles to the line of growth and the cut surface was polished, then the pattern could be seen as a slightly lighter area on the polished surface.

Of course the easiest and best way to cut and grind the shell is by use of a lapidary wheel, but since the substance of the shell is not hard it takes very little work to do the grinding by hand. This can be done very easily on a piece of glass with emery and water. It is a good plan to have two pieces of glass, one to use with coarse emery for rough grinding and another to use with fine emery to smooth the surface for polishing. The polishing is done on a fine stone such as is used for sharpening tools.

The cut should be made so that it shows both the inner and the outer lamina of the shell and the outline of some of the parietal tubes.

If the specimen that is being examined is small or delicate it may be difficult to hold during the cutting and polishing. A good way to handle such a specimen is to embed it in a small block of plaster of Paris. To make a mold for the block cut a strip of tin the width of the thickness of the block required and about six inches long, bend it into the shape desired, and put a rubber band around it. The inside of the mold should be greased before the plaster is placed in it. Set the mold on something to which the plaster will not stick, such as a piece of glass or tin, and fill the mold with plaster. Before the plaster hardens, press the shell in just level with the surface and with the bottom upturned. If the whole shell is to be worked it should be filled with plaster as this will keep it from being shattered in the grinding. When the block is ground it will be found that the plaster is ground more quickly than the shell; this leaves the shell projecting a little above the surface of the plaster, and this is just right for polishing. *Most patterns will completely disappear if the shell dries*, but wetting with water will make them appear again.

Dr. J. P. Harding of the British Museum in working with the patterns in the shell found that if the polished surface was moistened with benzyl alcohol the patterns became more clearly visible. This is very effective in some cases, but not in others. In examining *Balanus laevis nitidus* (Figs. 6, 7) for the first time I could see only the white dots, but the application of benzyl alcohol made the stem and branches also visible.

About fifty magnifications is enough to show up most of the patterns. Some few need more.

Discussion

By using the pattern, the sessile barnacle shell becomes a unique fossil for as far as is known it is the only shell that can be determined by examining a small fragment. If the shell is embedded in hard matrix it can usually be ground so that the base of one or more wall plates can be exposed to show any patterns.

The patterns in the wall plates extend from the basal edge that joins with the base right up to the apex. This can be seen by making several sections at different heights in a wall plate.

When enough patterns are illustrated it will probably be found that they can be divided into several classes. For instance *Balanus psittacus* (Figs. 31, 32) has a pattern that looks as if it might be the reflection of a plant. Then there is the teardrop pattern of *B. improvisus* (Figs. 1–3), *B. hesperius* form *laevidomus* (Figs. 19, 20), and *B. evermanni* (Figs. 37, 38).

Of all the species examined only two have patterns that are almost alike, *Balanus crenatus* (Figs. 34–36) and *B. concavus* (Figs. 46–49). However, the arrangement of the parietal pores differs. If the whole shell of *B. crenatus* is ground so that the basal ends of the wall plates are exposed it will be seen that the carinal margin of each plate projects more than the ribs. This is common in this species but rare in others. In both recent and fossil *B. crenatus* there is often an oblong mark between the patterns, as shown in Fig. 36.

Balanus balanus (Figs. 21, 22) has a most peculiar irregular pattern. The main stem is very dark. No other species that I have examined has a dark area in the pattern. The irregular white dots on each side of the dark line give it a most distinctive appearance.

A section of the shell of *Balanus trigonus* (Figs. 29, 30) shows the pattern projecting into, or forming, the rib. The scuta of this species have several rows of holes, or depressions, running from the basal margin nearly to the apex. *Balanus tintinnabulum californicus* (Figs. 8, 10) has a large and distinctive pattern. Another section of the same shell shows the effect of distortion (Fig. 9).

The pattern in the shell of *Balanus amphitrite* (Figs. 11–18) is a good example of the permanency of a pattern. The same pattern is found in specimens from widely separated localities and also in a fossil shell from Coraline Crag of England (Fig. 15). The profound change in the position and shape of the pattern and the shape and size of the parietal pores caused by distortion in different parts of the shell is shown in Fig. 18.

Some time ago I received some small fragments of barnacle shell and ground them to see the patterns. One is of special interest as the pattern is the same as that of *Balanus nubilus* (Fig. 39). Not only is the pattern the same as *nubilus* but the fragment is worn in the same way as the shell of that species is worn at the present time, that is with the outer lamina and most of the parietal tubes worn off, as shown in Fig. 41. *B. nubilus* is found only on the Pacific Coast of North America at the present time, but the fragment was from a Hungarian Miocene fossil bed. This is one example where it can be shown that the pattern of a species is unchanged through many ages. Other examples of this are: *B. concavus* now living on the California coast, and found in the Miocene fossil beds at Calvert Beach, Calvert County, Maryland; *B. nubilus* found in the Oligocene fossil beds of the Sooke formation on Vancouver Island, B.C.

The pattern and the outline of the parietal tubes may be much altered by distortion, and in making an examination of the shell there are several causes of variation that must be taken into consideration. First and most important

is the great adaptability of the shell shown by the tendency to take any form when crowded. For instance *Balanus nubilus* (Figs. 39–42) deepens its base when crowded, and this carries the body chamber out beyond the group where it can develop normally. Several intertidal species lengthen the wall plates when crowded. *B. crenatus* (Fig. 34) and *B. glandula* (Figs. 27, 28) both do this. The distortion evident in the bent patterns of *B. tintinnabulum californicus* (Fig. 9), *B. nigrescens* (Fig. 24), and *B. altissimus* (Fig. 26), shows what profound changes are made even in different parts of the same shell. In the normal pattern of *B. altissimus* (Fig. 25) the center line is zigzag between the branches. In *B. nigrescens* (Fig. 23) the center line is straight.

The pattern in the shell of *B. calidus* (Fig. 33) is the only one that is symmetrical. The pores are small and nearly round.

Species of barnacles have very definite vertical ranges, as for instance: *Balanus nigrescens* (Figs. 23, 24), which is found above low tide mark; *B. eburneus* (Figs. 43–45) found from low tide mark to 20 fathoms; *B. hameri* (Figs. 4, 5), which has been dredged from 16 to 167 fathoms; *B. evermanni* (Figs. 37, 38) dredged from 76 to 248 fathoms.

According to Withers (5) remains of pedunculate barnacles were first found in the Triassic rocks and the first sessile barnacles appeared in the Cretaceous rocks. Many species must have been developed and disappeared since that time and it seems probable that many patterns will be found in fossils that have no counterpart in living species.

Some patterns from fossil barnacle shells are shown by Cornwall (2).

Several subspecies have been examined and the difference between the pattern in a subspecies and the species does not seem any greater than the variation found in different parts of the same shell.

It seems likely that the patterns found in the shell may be formed by the growth of the corrugations that lock the wallplates to the base. If the shell is taken apart in such a way that the corrugations on the basal end of the wallplate are exposed and these corrugations are ground down it will be seen that the pattern of them is the same as the pattern in the wallplate (2).

The patterns described in this paper are from barnacles belonging to the genus *Balanus*, but they have been found in other genera. Davadie-Suaudeau (3) in her work on the fossil barnacles of Algeria shows the patterns of several genera. (It was this work that first started me on this investigation.) Gruvel (4) in his monograph shows the patterns found in several genera.

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Explanation of Figures

Fig. 1. *Balanus improvisus* Darwin. From Esquimalt Lagoon, Vancouver Island, B.C. Collected and identified by I. E. Cornwall. The pattern is the same as in U.S. National Museum specimen No. 51788 from Hecate Strait, B.C. Identified by H. A. Pilsbry.

Figs. 2, 3. Sections of shell 1 to show effect of distortion.

Fig. 4. *B. hameri* (Ascanius). From Point Prim, 10 miles west of Digby, N.S., from 46 fathoms. Collected and identified by E. L. Bousfield.

Fig. 5. Another section from shell 4. The patterns are faint.

Fig. 6. *B. laevis nitidus* Darwin. U. S. National Museum specimen No. 9224, from Peru. The white dots vary greatly in number and position in the same shell. Identified by H. A. Pilsbry.

Fig. 7. The same species as 6. U. S. National Museum specimen No. 131571, from Callo, Peru. Collected by Waldo L. Schmitt. Identified by I. E. Cornwall.

Fig. 8. *B. tintinnabulum californicus* Pilsbry. From Pacific Grove, California. From a little above low tide mark. Specimen loaned by Provincial Museum, Victoria, B.C. Identified by I. E. Cornwall.

Fig. 9. Another section from shell 8 to show distortion.

Fig. 10. A small specimen of No. 8 from La Jolla, Calif. Collected by Waldo L. Schmitt. Identified by I. E. Cornwall.

Fig. 11. *B. amphitrite* Darwin. From Newport Bay, Calif. Collected by P. Pickens. Identified by I. E. Cornwall.

Fig. 12. Another section of shell 11.

Fig. 13. *B. amphitrite* from Berkeley Aquatic park. Identified by I. E. Cornwall.

Fig. 14. *B. amphitrite albicostatus* Pilsbry. From coast of Japan. U.S. National Museum specimen No. 51790. Identified by H. A. Pilsbry.

Fig. 15. *B. amphitrite* fossil, from Low Pliocene, Plaisacian, Coraline Crag, England. British Museum specimen. Identified by I. E. Cornwall.

Fig. 16. *B. amphitrite*. U.S. National Museum specimen No. 6478, from Sarasota Bay, Fla. Identified by H. A. Pilsbry.

Fig. 17. *B. amphitrite* from Knysna, South Africa, collected by J. L. B. Smith of the Rhodes University, Grahamstown, South Africa. This section shows a normal pattern. Identified by I. E. Cornwall.

Fig. 18. Another section of shell 17 to show distorted pattern.

Fig. 19. *B. hesperius* form *laevidomus* Pilsbry from Esquimalt Lagoon, Vancouver Island, B.C. This has the same pattern as U.S. National Museum specimen No. 87691 from Hecate Strait, B.C. Identified by H. A. Pilsbry.

Fig. 20. Another section of shell 19 to show effect of distortion.

Fig. 21. *Balanus balanus* (Linnaeus) dredged from 46 fathoms at Point Prim, 10 miles west of Digby, N.S. Collected and identified by E. L. Bousfield.

Fig. 22. *B. balanus*. U.S. National Museum specimen No. 12077. No locality given with this specimen. Identified by I. E. Cornwall. U.S.N.M. specimen No. 69588 from North Greenland has the same well-marked black lines. Identified by C. R. Shoemaker. As far as is known this is the only barnacle that has black lines in the figure. The figures from different parts of one shell vary greatly, and no typical figure could be found. The black line is straight in one place and zigzag in another part of the same shell, but there are always several light marks on each side of it.

Fig. 23. *B. nigrescens* Lamark from Wilson's Promontory, Victoria, Australia. Collected by Miss E. Pope of the Australian Museum, Sydney, Australia. Identified by I. E. Cornwall.

Fig. 24. Another section of shell 23 to show effect of distortion. The pattern in this species has a resemblance to the pattern in *B. altissimus*, Fig. 25, but the branches of the pattern in Fig. 23 are opposite, those in Fig. 25 are alternate and never divided like some in 23. Both 23 and 25 are found in the intertidal zone.

Fig. 25. *B. altissimus* Cornwall from William Head, Vancouver Island, British Columbia. Found only above low tide mark. Drawings made from type loaned by the Provincial Museum, Victoria, B.C. Identified by I. E. Cornwall.

Fig. 26. Another section of shell 25 to show effect of distortion.

Fig. 27. *B. glandula* Darwin from Cordova Bay, Vancouver Island, B.C. The small round pores are filled with white powder. Identified by I. E. Cornwall.

Fig. 28. Another part of the shell 27 to show distortion. Nos. 27 and 28 are both drawn to same scale.

Fig. 29. *B. trigonus* Darwin. U.S.N.M. specimen No. 85956 from outside Batia Columbia. Collected by Waldo L. Schmitt from 8 to 9 fathoms. The shape of the parietal pores varies greatly in different parts of the same shell. Identified by H. A. Pilsbry.

Fig. 30. *B. trigonus*. U.S.N.M. specimen No. 63372, from Panama. Identified by H. A. Pilsbry.

FIG. 31. *B. psittacus* (Molina). U.S.N.M. specimen No. 89298, from Antafagasta, Chili. The size and shape of the parietal pores vary greatly in different parts of the shell. Identified by G. P. Ashcraft.

FIG. 32. *B. psittacus*. U.S.N.M. specimen No. 49549, from Chincha Island, Peru. Identified by H. A. Pilsbry.

FIG. 33. *B. caladus* Pilsbry. U.S.N.M. specimen No. 10069, from 27 fathoms in Gulf of Mexico. Identified by I. E. Cornwall by coverplates and shell only as body was missing.

FIG. 34. *B. crenatus* Bruguiere. U.S.N.M. specimen No. 11161, Albatross Station No. 2448. Identified by H. A. Pilsbry. The same pattern was found in many specimens from numerous places on the coast of British Columbia.

FIG. 35. Another section of shell 34 to show variation in shape of pores.

FIG. 36. Fossil *B. crenatus* from glacial deposit near Jordan River, Vancouver Island, B.C. Identified by I. E. Cornwall.

FIG. 37. *B. evermanni* Pilsbry. U.S.N.M. specimen No. 52133, Albatross Station 3602. The internal ribs vary in different parts of the shell. Identified by H. A. Pilsbry.

FIG. 38. A section from another part of shell 37.

FIG. 39. *B. nubilus* Darwin. From William Head, Vancouver Island, B.C. Identified by I. E. Cornwall.

FIG. 40. *B. nubilus*. U.S.N.M. specimen No. 61602, from Pacific Grove, Calif. Identified by H. A. Pilsbry.

FIG. 41. *B. nubilus*. Fossil, from Oligocene Sooke formation, Vancouver Island, B.C. Identified by I. E. Cornwall.

FIG. 42. Fragment of *B. nubilus* shell from Hungarian fossil beds. Identified by I. E. Cornwall.

FIG. 43. *B. eburneus* Gould. U.S.N.M. specimen No. 10753, from Woods Hole, Mass. Identified by H. A. Pilsbry.

FIG. 44. Another part of shell 43.

FIG. 45. *B. eburneus*. U.S.N.M. specimen No. 51771, from Vineyard Sound. Identified by H. A. Pilsbry.

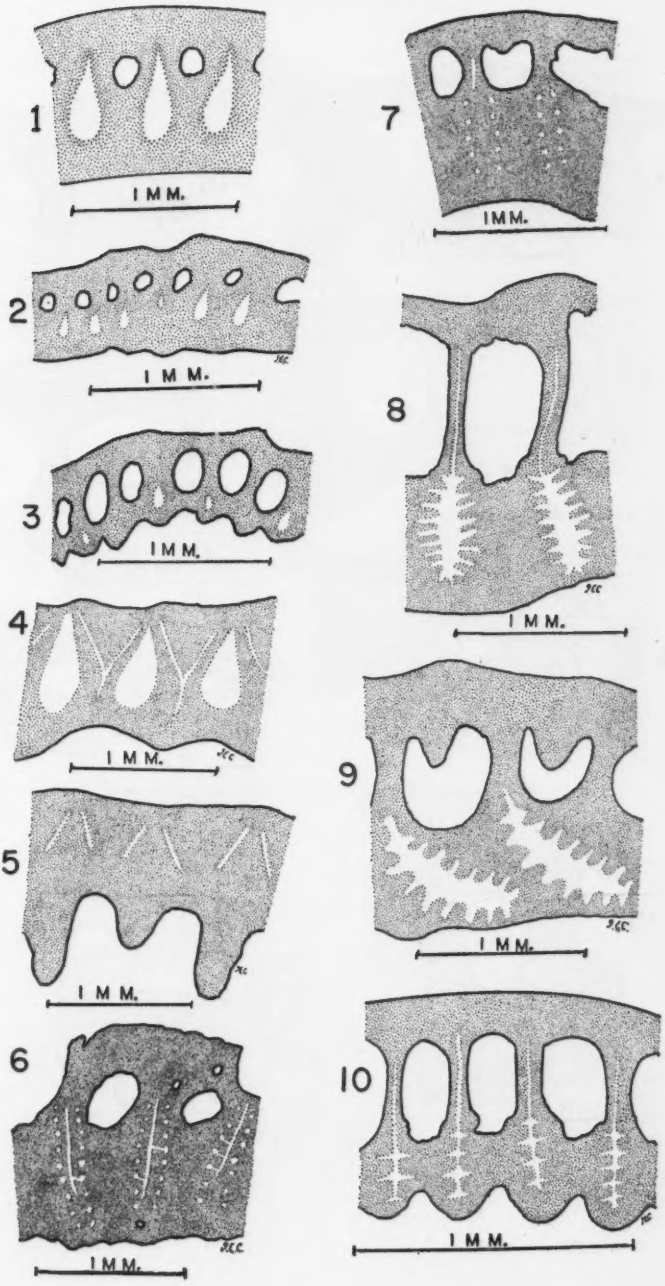
FIG. 46. *B. concavus* Bronn. Fossil from Miocene formation, one-half mile north of Scientist Cliff, Chesapeake Bay, Md. Identified by I. E. Cornwall.

FIG. 47. *B. concavus pacificus* Pilsbry. U.S.N.M. specimen No. 87647, from Santa Maria Beach, Calif. This section was made near the base of the wallplates and shows the pattern projecting into the lower part of the ribs. Collected and identified by D. P. Henry.

FIG. 48. *B. concavus pacificus*. U.S.N.M. specimen No. 51682, from Long Beach, Calif. Identified by H. A. Pilsbry.

FIG. 49. *B. concavus*. Fossil from same place as 46. Identified by I. E. Cornwall.

NOTE: Figs. 1–49 follow.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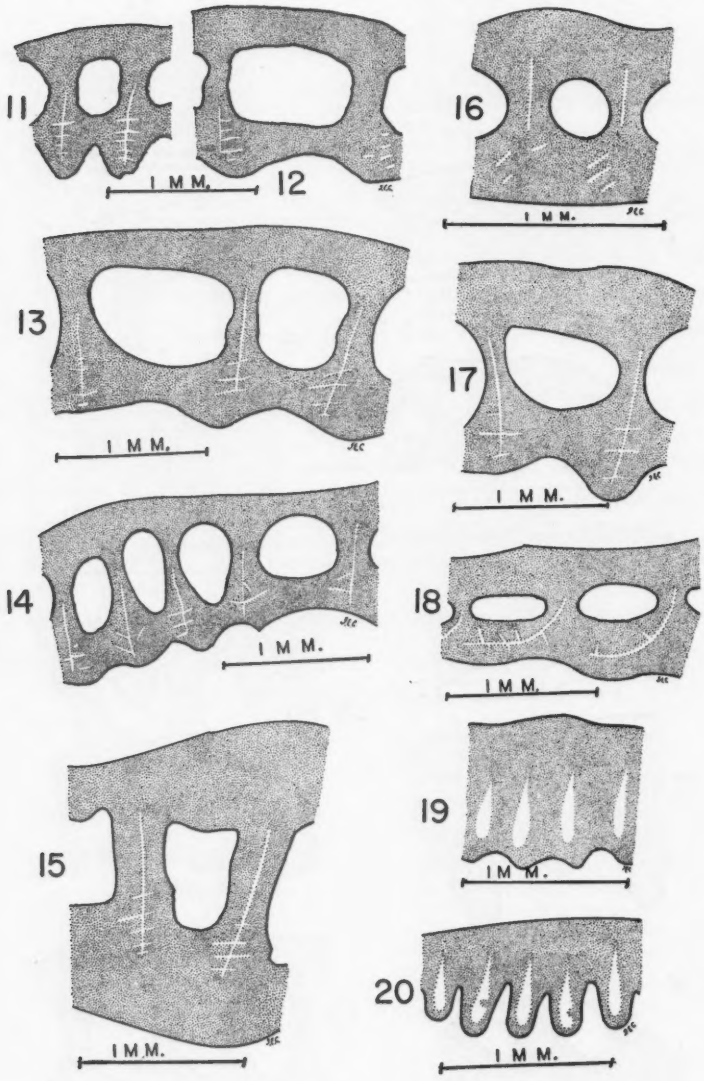
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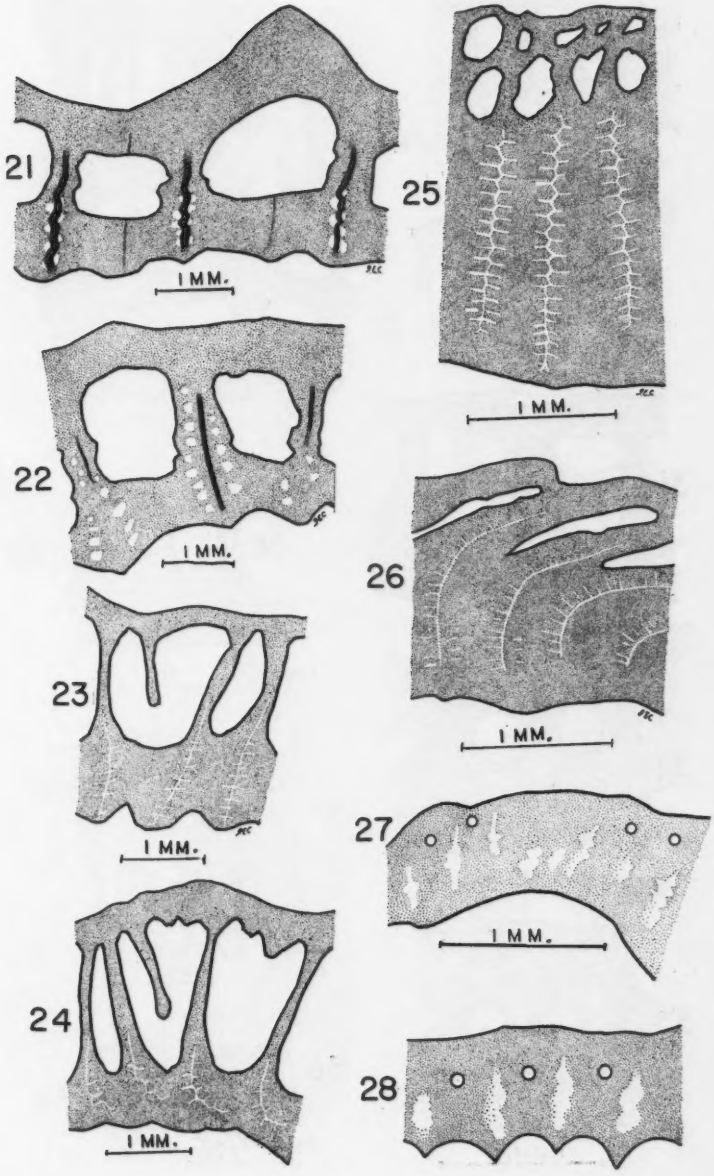
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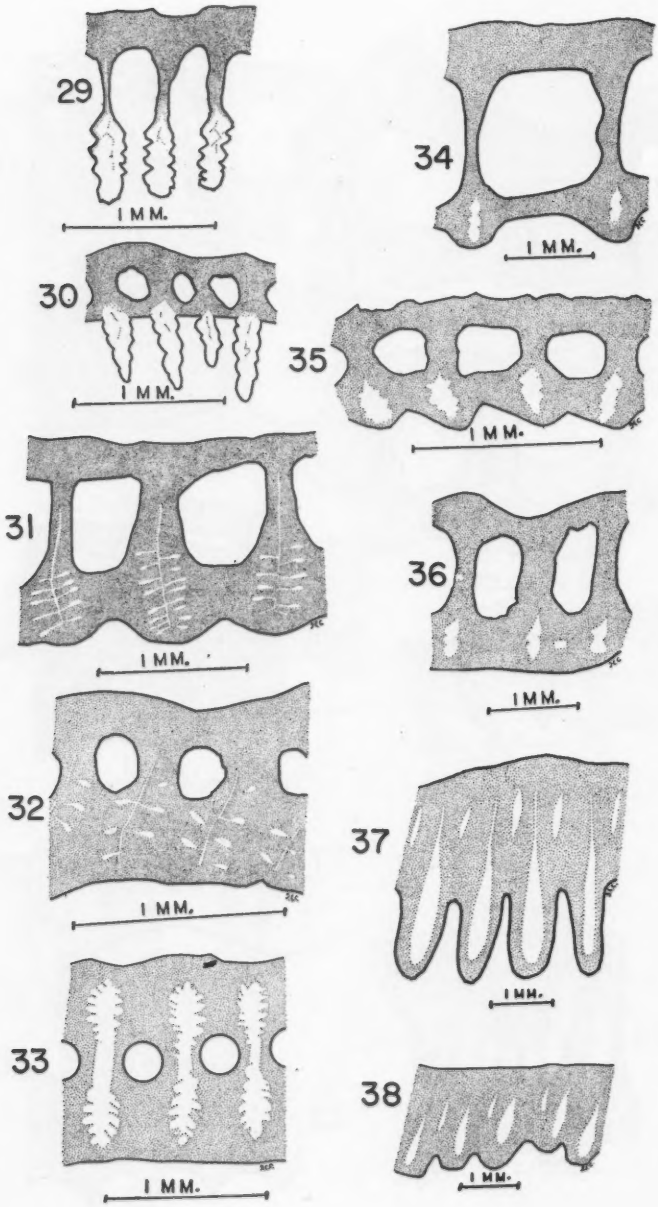
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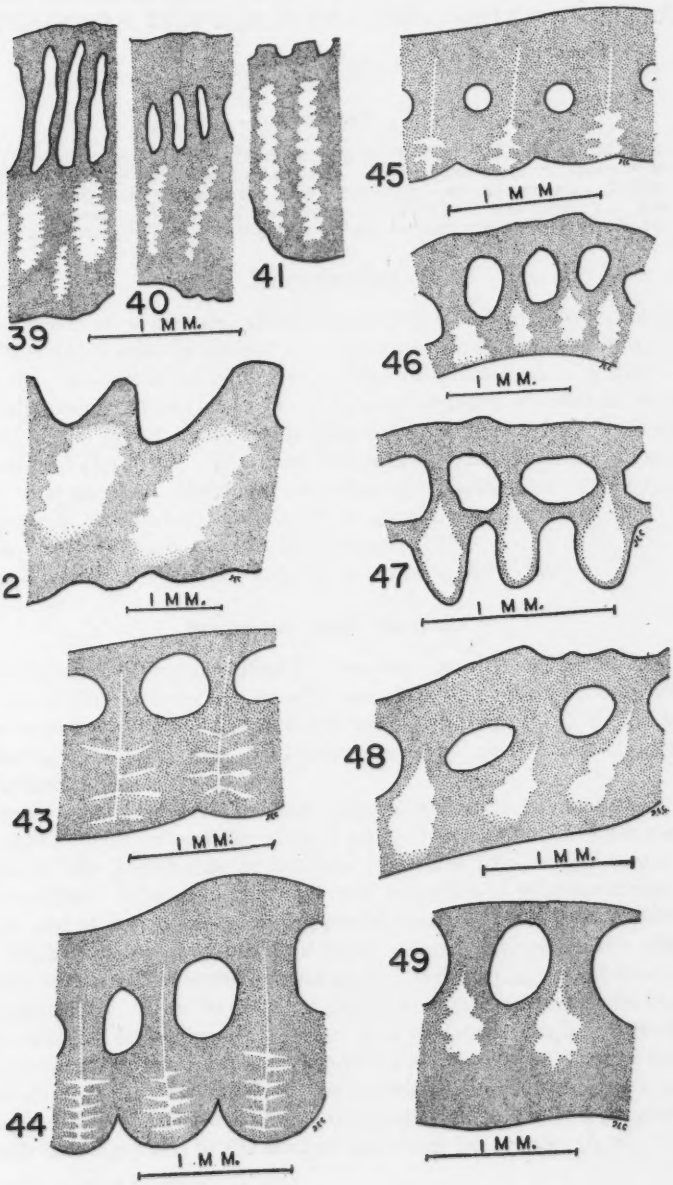
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PARTHENOGENESIS IN A CYST-FORMING NEMATODE, HETERODERA TRIFOLII (NEMATODA: HETERODERIDAE)[1]

ROLAND H. MULVEY[2]

Abstract

The clover cyst nematode, *Heterodera trifolii* (Goffart, 1932) Raski and Hart, 1953, reproduced in the absence of males. Under greenhouse conditions nematodes reared from single larvae and from mass cyst culture were diploid-parthenogenetic. During maturation only one polar body was produced. The diploid number (24 ?) of chromosomes was not reduced and no male was found.

Introduction

The clover cyst nematode, *Heterodera trifolii* (Goffart, 1932) Raski and Hart, 1953, which attacks several cultivated legumes, is fairly widespread in Canada (7). Examination of a large volume of infected plant material from the greenhouse at Ottawa and from fields near Ottawa revealed no males on the roots. This agrees with Hirschmann (4), Gerdemann and Lindford (3), and Raski and Hart (10). However, Franklin (2) and McBeth (5) described males of this species. Males are abundant in populations of the soybean cyst nematode, *H. glycines* Ichinoe, 1952, a closely related form, and also in the sugar-beet nematode, *H. schachtii* Schmidt, 1871.

This is a report on oogenesis and reproduction in *H. trifolii*.

Materials and Methods

The material used was identified by the writer as of *H. trifolii* by host preference (white Dutch clover was heavily attacked, and extensive tests showed that the nematode would not attack red beet or rutabaga root), by the yellow phase during cyst formation, by fenestral length, and by underbridge size and structure.

An ample supply of gravid white females was made available for mass cultures of the nematode on the roots of white Dutch clover, *Trifolium repens* L., grown in the greenhouse at Ottawa. Cultures from single larvae were reared as follows: White Dutch clover seed was sown in flats containing sterilized soil, and after a 2-week growing period single plants were transferred to 250-ml. beakers containing sterilized sand. Each plant was then carefully inoculated with a single active second-stage larva by placing the larva close to the plant roots. After 10 days the plants were removed from the beakers, carefully washed under running water, and replanted singly in glass jars (250 ml.) containing vermiculite. A nutrient solution was added to each jar to maintain growth. The level of this solution was maintained by adding solution as required. The live female nematodes were prepared for study by the squash technique and in the manner described by Mulvey (6, 8).

[1]Manuscript received September 24, 1957.
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Oogonial Development

Oogonial development follows closely that described by Mulvey (6) for a species of *Heterodera* (probably *H. trifolii*) on hairy vetch (*Vicia villosa* Huds.), except that no prochromosomes were observed.

Oocytes

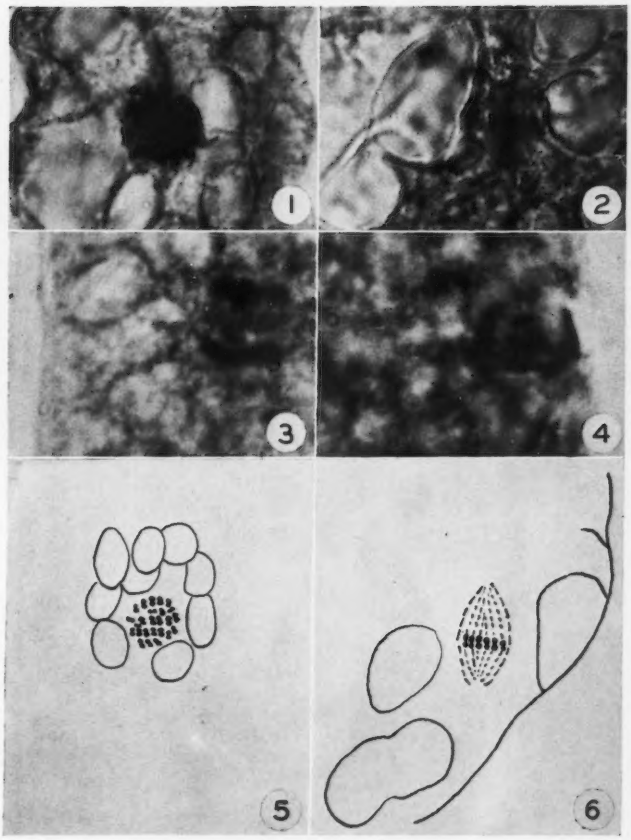
Oocytal development also follows closely that described by Mulvey (6) in the species of *Heterodera* on hairy vetch. After completion of the growth period the chromatin is organized into discernible chromosomes. Duplication of each chromosome becomes discernible with no pairing of the homologous chromosomes. Therefore, only the dyad condition is reached (Figs. 1, 2, 5, 6). Mulvey (8) reported that the homologous chromosomes pair in the females of the bisexual nematode *H. schachtii*. The achromatic material is well defined (Fig. 2) and the cytoplasm of the egg densely vacuolated. During early metaphase the spindle and its nuclear elements lie at the center of the egg. Eventually the chromosomes separate (Fig. 3) and the spindle, which is normally parallel to the long axis of the egg, rotates through an angle of 90° and comes to rest with one of its poles against the cell membrane (Fig. 4). Only one polar body is produced and the diploid number of chromosomes is not reduced. The chromosomes are very small and, therefore, accurate counting was not possible. However, the diploid number appears to be at least 24.

Although many females were examined no sperm was found. Cultures from single larvae, each of which developed into a gravid female in the absence of males, in turn produced gravid females. Second-generation females contained no sperms.

Discussion

These studies indicate that *H. trifolii* is capable of reproducing parthenogenetically, at least under greenhouse conditions. According to Walton (11), only in rare cases does the nematode egg develop without fertilization by the sperm. Belar (1) reported that two parthenogenetic species of *Rhabditis* show a single maturation division and no reduction in the chromosome number. Mulvey (6) described a species of *Heterodera* in which the chromosome number was not reduced during maturation division but he did not observe polar body formation. Hertwig (cited by Walton (11, p. 208)), investigating a dioecious culture of *Rhabditis pellio* (Schneider, 1866) Butschli, 1873, found a mutant that produced only one polar cell without reduction and, therefore, retained the diploid number of 14 chromosomes. None of the eggs developed without sperm being present in the egg although the sperm did not enter the cleavage nucleus. Nigon (9) reported that in the free-living nematode *Rhabditis bellari* Nigon, 1949 some eggs undergo two reductional divisions and their pronuclei fuse; these produce males and females. Other eggs, which produce only females, form a single polar body; at the same time the bivalents divide twice and in these pseudogamous eggs development proceeds from the diploid female nucleus, the sperm remaining inert.

PLATE I



FIGS. 1–4. Photomicrographs showing maturation stage in a cyst-forming nematode, *Heterodera trifolii*. 1500×

1. Polar view of early metaphase stage in primary oocyte showing dyads. 2. Side view of metaphase stage showing achromatic material and densely vacuolated cytoplasm of the primary oocyte. 3. Side view of early anaphase stage in primary oocyte. 4. Side view of late anaphase stage of primary oocyte. The polar body is produced at this stage.

FIGS. 5–6. Camera lucida drawings showing finer details in Figs. 1 and 2. 1500×

Acknowledgments

The author is indebted to Mr. A. L. Sauve, greenhouseman, for his help in propagating and maintaining plants during the study.

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An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

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